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ANNUAL REPORT OF RESEARCH

OCTOBER 1, 1991 through SEPTEMBER 30, 1992

DIVISION OF VIROLOGY

FOOD AND DRUG ADMINISTRATION

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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DIVISION OF VIROLOGY
FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

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DIRECTOR'S SUMMARY

DIRECTOR'S SUMMARY

DIVISION OF VIROLOGY

Annual Report for October 1, 1991 through September 30, 1992

In the FY1991-1992 the Division of Virology was instrumental in developing the new Laboratory of Vector-borne Viral Diseases. This new Laboratory is headed by Dr. Lewis Markoff who came to the Food and Drug from the National Institutes of Health. This Laboratory was designed specifically to research the viruses of the family flaviviridae, with emphasis on the dengue (DEN) viruses, and the Japanese encephalitis virus (JEV). A future objective of this Laboratory will include research of the hepatitis C virus. All three of these viruses cause diseases of major significance. Along with research, the Laboratory of Vector-borne Viral Diseases will perform regulatory duties for biological products relating to viruses and to direct the course of research on epidemiologically important vector-borne viruses.

Successively, in the Laboratory of Hepatitis two major projects of research involving the hepatitis A virus (HAV) and hepatitis C virus (HIV) were developed. These projects were found to have many important implications in providing basic information toward identification and characterization of viral replication and pathogenesis. In the HAV project, the identification of the cellular receptor may permit development of a small animal model which would facilitate the research in the areas of pathogenesis and vaccinology, and may prove useful for evaluating licensed vaccines for safety and potency. Equally broad ranging in its implications, is the study of the HCV research. The HCV research has begun a process of construction of full length clones of the HCV genome to evaluate them for infectivity. If these clones prove to be infectious it will permit genetic manipulation that may result in necessary characteristics for a vaccine. For more information regarding this work reference the Laboratory Summary of Dr. Feinstone.

In the area of retrovirology, research was augmented under the direction of Dr. Klinman. This research focused on three major areas relating to HIV infection. The first considered the regulation of the humoral immune response to HIV-1 envelope glycoproteins. Secondly, two murine models of HIV infection were examined. The effect of immunization on tolerance and disease progression was investigated in transgenic mice; and the SCID mouse model allowed to analyze the degree of protection conferred by

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

various immunization regimens. Significantly, an outgrowth of this research has been the finding that anti-HIV reactive cells are present initially in peripheral circulation and then home to the bone marrow of immunized donors. The third area of research work provided considerable insight into the degree to which HIV-1 specific B cells are activated in patients with ARC and AIDS.

Dr. Arifa Khan studied the genetic relationship of primate lentiviruses in HIV-1, HIV-2 and SIV. Fragments obtained from all strains were cloned to determine evolutionally relatedness of these endogenous sequences. Another project centered on the development of a primate HIV model using pig-tail (macaque) monkeys to vaccinate and to show protection against the prevalent MN strain of HIV-1.

Dr. Quinnan, Ms. Vujcic and Ms. Glass researched three major areas of HIV infection: 1) Sequential human sera were tested for neutralizing antibodies against autologous HIV-1 isolates; 2) They prepared HIV-1 reference reagents for the World Health Organization and, initiated an international collaborative test study; six laboratories in Europe and the United States are involved in this research. 3) V3 loop region synthetic peptides were constructed as blocking agents in an HIV-1 neutralization assay.

A continuing objective for the Laboratory of Pediatric Diseases is to find a standard reproducible method for determining the potency of inactivated poliovirus vaccines (IPV) in vitro to advance the eradication of polio. Dr. Sawyer spearheaded several projects in the Laboratory that dealt with improving techniques to measure the potency of IPV. One outgrowth was the organization of an International Collaborative study under the auspices of WHO for standardizing the ELISA test for IPV potency measurement.

In addition to these studies, Dr. Albrecht worked closely with the Center for Disease Control investigating the serological response of US children to two measles virus vaccines. One vaccine was US-licensed and the other, an experimental vaccine identified as Edmonston-Zagreb. Although the experimental vaccine was more immunogenic (when used in a tenfold higher concentration), this dosage is not recommended for routine use.

Dr. Lundquist initiated a study of live poliovirus vaccine as to how it can be lyophilized to improve conditions for its storage. This should improve the cold chain facilitate immunization programs. A significant step was achieved preserving 30-40% of viral infectivity on lyophilization. Another project Dr.Lundquist pursued was an in vitro polio translation system based on Hela cell extracts to determine and compare characteristics of the transcription of wild type and subsequently attenuated type viral RNA.

In collaboration with a number of laboratories requiring expert electron microscopy evaluation, Dr. Jackie Muller performed three major studies. Examined and evaluated for morphological evidence of infection were the indentification of macrophage-like

cells in peritoneal fluid of mice with MAIDS syndrome, U937 cells, and liver biopsies of chimpanzees infected with hepatitis C.

Finally, the Laboratory of Pediatric Diseases was involved in providing training in vaccine potency testing and viral antibody measurement to individuals from several countries. For further details reference the last page of the Pediatrics Summary.

In the Laboratory of Respiratory Virus Diseases Dr. Beeler expanded her studies on the antigenic composition of the respiratory syncytial virus F glycoprotein. She centered her study around the RSV-F by developing an antigenic site blocking assay. This assay holds much promise for further studies that will allow comparisons of immune responses to the F glycoprotein of individuals have been naturally infected or immunized.

The most accomplished project by Dr. Levandowski was the development of a PCR assay which resulted in a more sensitive method of detection of genetically mixed influenza virus populations. These PCR techniques can be extremely useful in the production of reassortant viruses for use in the production of inactivated vaccine.

Highly relevant was Dr. Levandowski's research as to the response of infants and toddlers to influenza virus infection. Good evidence was presented that killed influenza vaccine will not induce sustained protection, and therefore the first exposure in this young age group should be to live influenza vaccine.

The Laboratory of DNA Virus Research under the direction of Dr.Carol Marcus-Sekura, continued a number of related projects designed to analyze immune responses to recombinant HIV proteins and to study the properties of the recombinant antigens.
Dr. Marcus-Sekura continued regulatory work by attempting to answer many questions posed by use of specific cell lines for their suitability for production of biological products. Both Dr.Marcus-Sekura and Dr. Zhang, a visiting scientist in her lab, continue to assess the potential of EBV gp350 as an EBV vaccine.

Dr. Hana Golding continued several projects relating to immunopathological aspects of HIV-1 infection and the anti-HIV therapeutic CD4. A detailed description of all these related projects and their important findings can be identified in the Laboratory Summary.

The Oncology Group, headed by Dr. Abdur Razzaque, continued two major projects of the herpesviruses. Dr. Razzaque, in collaboration with others, continues to examine the oncogenic potenial of the human herpesviruses, CMV and HHV-6, the latter of which was recently identified in AIDS patients and patients with lymphoproliferative disorders.

The Unit on Latency, a recent addition to the Laboratory of DNA Virus, is headed by Dr. Philip Krause. Dr. Krause plans to

continue the work on HSV latency begun at NIAID/NIH by investigating the role, regulation and mechanism of action of HSV latency-associated transcripts (LATs). For details read the section Unit on Latency in the Laboratory Summary.

Further Clinical Programs on CMV and EBV conducted respectively by Ms. Manischewitz and Mr. Armstrong are cited in the Laboratory Summary. These Clinical Programs are both involved in monitoring patient specimens for effects and therapy in a series of clinical trials; both in conjunction with other noted sources.

The Division of Virology reviewed in 1991-1992 623 original IND submissions and supplements of these 147 were AIDS-related. In addition, the Division reviewed 110 PLA/ELA original submissions and supplements. There were 45 amendments for licensed products submitted for review by the Division, and the Division participated in the review of 67 product labeling submissions.

This year the Division was actively involved in the review of vaccine labeling and package circulars as mandated by Section 314 of the National Childhood Vaccine Injury Act.

The Division was very active in pursuing licensure of Japanese Encephalitis vaccine resolving issues relating to allergic reactions following vaccination and product characterization requirements.

Members of the Division of Virology were invited as speakers or session chairpersons at international, national and local meetings in the area of their expertise. The following persons and their engagements are cited below:

Paul Albrecht, MD, Director of Division of Virology

PRESENTATIONS AND MEETINGS

Invited Participant, WHO Informal Discussion on Polio Neutralizing Antibody Tests, Vanderbilt University, Nashville, TN, December 5-6, 1991

Invited Participant, Consultative Meeting on Measles Laboratory Diagnosis; Sponsor PAHO, Washington DC; February 2, 1992

Invited Participant, WHO Consultation on Studies Involving High Titer Measles Vaccine before Nine Months of Age, Atlanta GA, June 16-17, 1992

Invited Participant, Measles Diagnostics Meeting; Sponsor: The Task Force for Child Survival and Development, Atlanta, GA; June 18, 1992

Panelist, International Meetings on Spongiform

Encephalopathics, Heidelberg, Germany June 23-24, 1992

Invited Participant, Measles Workshop; Sponsor: DMID/NIAID/NIH, Holiday Inn Crown Plaza, Rockville MD, September 14-15, 1992

Carol J. Marcus-Sekura, PhD, Acting Deputy Director, Division of Virology

PROFESSIONAL PRESENTATIONS

International workshop on "Preclinical Safety Testing of Monoclonal Antibodies" held January 9-10, 1992. Co-Chairman of session of adventitious agents and presentation on "Retrovirus Contamination: Summary of Recent Meetings".

Invited participant in a panel discussion on "Virus Validation" at BioEast Symposium held in January 1992.

Carol J. Marcus-Sekura, Amy M. Woerner, and Michael Klutch, Characterization of a DNA Binding Domain at the C-Terminus of HIV-1 Integrase at Cold Spring Harbor Meeting on RNA Tumor Viruses, May 19-24, 1992.

Amy Woerner, Michael Klutch, and Carol Marcus-Sekura, Deletion Analysis of a DNA Binding Domain at the C-Terminus of HIV-1 Integrase at NIH Research Festival, September 21-22, 1992.

Peng-fei Zhang and Carol Marcus-Sekura, Effect of Glycosylation of Recombinant EBV gp350 on Recognition by a Panel of Monoclonal Antibodies, NIH Research Festival, September 21-22, 1992.

COMMITTEES

Editorial Board, Analytical Biochemistry

Editorial Board, Antisense Research and Development

Center for Biologics Committee to Revise the Cell Substrate "Points to Consider Document"

Center for Biologics Committee to Revise the Monoclonal Antibody "Points to Consider Document"

Center for Biologics Genetic Stability Task Force

Center for Biologics Monoclonal Antibody Workshop Planning Committee - planned agenda for meeting held in January 1992

Center for Biologics Promotions and Tenure Committee - agency committee which evaluates applications on scientific merit

and recommends candidates to the agency director and subsequently to the NIH scientific directors.

Center for Biologics Committee on Bioequivalency

Center Reorganization Task Force: Office of Vaccines

Dennis M. Klinman, MD, PhD, Laboratory of Retrovirus Research, Division of Virology

AWARDS: PHS Outstanding Service Medal, 1992

PROFESSIONAL PRESENTATIONS

Speaker, "B cell activation in SLE", Combined Clinical Conference on Systemic Lupus Erythematosus, Grand Rounds, NIH, 1991

Speaker, "B cell activation in HIV-1 infected patients", AIDS Vaccine Evaluation Group, Bethesda MD, 1991

Speaker, "An overview of vectors and potential problems in the use of viral vectors", International Workshop on Continuous Cell Lines, NIH, 1991

Speaker, "Comparative cross-reactivity of normal versus autoreactive B cells", FASEB Conference, Atlanta GA, 1991

Speaker, "Cell line issues related to specific expression vectors", Fifth ABC International Biotechnology Meeting, Washington DC, 1991

Speaker, "B cell activation in human and murine AIDS", Johns Hopkins University School of Public Health, Baltimore MD, 1991

Speaker, "Use of CD4 as a surrogate endpoint for evaluation of anti-HIV immunotherapeutics", CBER Vaccine Advisory Committee, Bethesda MD, 1991

Speaker, "B cell defects in SLE", NIAMS Scientific Workshop on SLE, NIH, 1992

Speaker, "Active and Passive Immunotherapy of HIV", VII International Conference on AIDS, Amsterdam, The Netherlands 1992

Speaker, "HIV-1 Therapeutic Vaccine Trials", NCVDG Workshop on Therapeutics Vaccines, Washington DC, 1992

COMMITTEES

Chairman, Session on Murine Models of SLE, UCLA Symposium on Self Reactivity and its Regulation, Keystone CO 1991

Hana Golding, PhD, Laboratory of DNA Virus Research, Division of Virology

INVITED PRESENTATIONS

Department of Pathology, Georgetown University
A lecture: "Reduced susceptibility to HIV-1 infection correlates with low levels of NFk-B DNA binding proteins in mutagenized CEM subclones"

Division of Retrovirology, Walter Reed Army Institute of Research
A lecture: "Molecular mimicry between HIV-1 gp 41 and human HLA class II antigens results in the generation of crossreactive autoantibodies with immunosuppressive potential early in the course of infection"

1992 International Congress of Immunology, Budapest, Hungary
Oral presentation at a workshop on HIV-1 pathogenesis: "Fusion of human B cell lines with HIV-1 expressing T cells can be enhanced by antigen-specific immunoglobulin receptors: Possible mechanism for elimination of gp120-specific B cells in vivo"

Frontiers in Infectious Diseases: Focus on HIV, 1992, Hertfordshire, England
A lecture: "Autoimmune response in HIV-1 infection"

MRC AIDS Workshop 1992, Edinburgh, Scotland, England
A lecture: "A new assay to study HIV-1 env-mediated cell fusion: The role of CD4 and the adhesion molecules LFA-1 during fusion and syncytia formation"

Leigh A. Sawyer, DVM, MPH, Laboratory of Pediatric Diseases, Division of Virology

AWARDS: PHS, The Achievement Medal, November 1991

PROFESSIONAL PRESENTATIONS

11th Annual Meeting, American Society of Virology, July 1992, Ithaca, New York, "Comparative Reactivity of Monoclonal and Polyclonal Antibodies in ELISA Tests of Inactivated Poliovirus (IPV) Vaccines"

Weekly Scientific Conference, Center for Biologics Evaluation and Research, FDA, February 27, 1992 Bethesda, MD, "Studies of Potency of IPV alone and in Combination with DTP or Thimerosal, using ELISA and Mice"

FDA Vaccines and Related Biological Products Advisory Committee, November 1991 Bethesda, MD, "Japanese Encephalitis Virus Vaccine, Issues of Safety, Purity, Potency and Efficacy"

COMMITTEES

Chairman, PLA Committee for Japanese Encephalitis Virus Vaccine, Inactivated

Chairman, ELA Committee for Japanese Encephalitis Virus Vaccine, Inactivated

Chairman, PLA Committee for Purified Chick Embryo Cell Rabies Vaccine

Member, ELA Committee for Purified Chick Embryo Cell Rabies Vaccine

Member, PLA Committee for Human Diploid Cell Rabies Vaccine

Chairman/Member of five different PLA Amendment Committees for Rabies Vaccine, Rabies Vaccine Absorbed, or Inactivated Poliovirus Vaccine

Member Working Group: Section 314 of the National Childhood Vaccine Injury Act, 1992

Member, Committee to review Request for Proposals for Microencapsulation of Pertussis Proteins

Abdur Razzaque, PhD, Laboratory of DNA Virus Research, Division of Virology

PROFESSIONAL PRESENTATIONS

Human Herpesvirus-6 Meeting, a Satellite Symposium to the XVII International Herpesvirus Workshop, August 1, 1992 Edinburgh, Scotland; titled "HHV-6 strain variation"

Human Herpesvirus-6 Meeting, a Satellite Symposium to the XVII International Herpesvirus Workshop, August 1, 1992, Edinburgh, Scotland; titled "Transforming function of HHV-6"

Sigma XI, Food and Drug Administration, Science Exposition, May 21-22, 1992; titled "Transforming DNA sequences in HHV-6 GD strain isolated from a patient with chronic fatigue syndrome"

Judy A. Beeler, MD, Laboratory of Respiratory Virus Disease, Division of Virology

PRESENTATIONS

Workshop to Review Warnings, Use Instructions, and Precautionary Information under Section 314 of the National Childhood Vaccine Injury Act, September 1992; "Labelling issues relevant to the use of live attenuated measles, mumps, and rubella vaccines"

Workshop to Review Warnings, Use Instructions, and Precautionary Information under Section 314 of the National Childhood Vaccine Injury Act, September 1992; "Labelling issues relevant to the use of poliovirus vaccines"

COMMITTEES

Member, Committee for Workshop under Section 314 to review package inserts for labelling of childhood vaccines

Chairman, PLA Committee to review the application for licensure of a live, attenuated trivalent oral poliovirus vaccine proposed by Connaught Laboratories Ltd, Canada

Lewis Markoff, MD, Laboratory of Vector-borne Viral Diseases,
Division of Virology

PRESENTATIONS

11th Annual Meeting, American Society of Virology, July 1992 Cornell University, Ithaca New York; Flavivirus Session, presented original work titled "Ubiquitination of the dengue virus capsid protein"

Chaired the Flavivirus session at the American Society of Virology meeting, Cornell University, Ithaca, New York, July 1992

COMMITTEES

Advisor, PLA Committee evaluating the JEV vaccine proposed by the Connaught Company in the U.S. representing BIKEN of Japan

Chairman, PLA Committee to review the application for licensure of a killed hepatitis A vaccine proposed by Smith, Kline, and French Labs

Barry Falgout, PhD, Laboratory of Vector-borne Viral Diseases,
Division of Virology

PRESENTATIONS

11th Annual Meeting, American Society of Virology, July 1992 Cornell University, Ithaca New York, Flavivirus Session,

presented original work titled "Deletion analysis of dengue type 4 virus nonstructural protein NS2B"

Arifa S. Khan, PhD, Laboratory of Retrovirus Research, Division of Virology

Speaker, Workshop on Pathogenesis by Non-Acute Retroviruses; Chase Cancer Center, Philadelphia, PA 1991

COMMITTEES:

PTC for Monoclonal Antibodies

PTC for Cell Substrate

Working Group on Retroviral-related Safety Issues

Team Member of Environmental Audit of Medical Pathological Waste at the NIH

Paul Albrecht

Paul Albrecht, M.D.
Director, Division of Virology

10/8/92
Date

PERSONNEL LIST

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH
DIVISION OF VIROLOGY

Director, Paul Albrecht, M.D.
Deputy Director, Acting, Carol J. Marcus-Sekura, Ph.D.

Senior Staff
Richard Daemer, Ph.D.
Barry Falgout, Ph.D.
Stephen M. Feinstone, M.D.
Hana Golding, Ph.D.
Arifa Khan, Ph.D.
Dennis Klinman, M.D., Ph.D.
Ronald E. Lundquist, Ph.D.
Lewis Markoff, M.D.
Jacqueline M. Muller, Ph.D.
Leigh A. Sawyer, D.V.M.

Fellows
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Audrey Chang, Ph.D.
Mark Cosentino, Ph.D.
Gerardo Kaplan, Ph.D.
Philip Krause, M.D.
Roland Levandowski, M.D.
Abdur Razzaque, Ph.D.
David B. Wilde, M.D., Ph.D.

Visiting Program
Toshitaka Akatsuka, M.D., Ph.D
Mikhail Donets, M.D., Ph.D.
Jiahua Qian, Ph.D.
Peng Fei Zhang, Ph.D.

Guest Researcher
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Andreas Dotzauer, Ph.D.
Valeria Tedeschi, Ph.D.

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Ichiro Aoki, M.D.
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Marina Zaitseva, Ph.D.

Laboratory Support Staff

Lab of DNA Virus Research
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- Michael Klutch
- Jody Manischewitz
- Jeffrey Smith
- Ollie Williams
- Amy M. Woerner
- Marina Zaitseva

Lab of Respiratory Virus Disease
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- Daniel Offringa
- Eldridge Staton
- Vicki Tyson-Medlock
- Michael Williams

Lab of Hepatitis Research
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- B.Richard Prather, Jr

Lab of Pediatric Diseases
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- John A. Cleveland
- Susette Fejerang
- Sandhya Kulkarni
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- Jonathan McInnis
- Amita Patel

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- Sylvester Daniel
- Teresa Galvin
- Wendy Glass
- Luba K. Vujcic

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- Christine Winter

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Regulatory Review Staff: Paula Lincoln-Smith

CONTRACT REPORTS

CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

CONTRACT REPORT
October 1, 1991 through September 30, 1992

Contractor: HACKENSACK HOSPITAL, HACKENSACK, NEW JERSEY

Title: Evaluation of Influenza Virus Vaccines in High Risk Infection Categories

Contractor's Project Director: Peter A. Gross, M.D.
(Contract Number: FDA 233-90-1102)

CBER Project Officer: Roland A. Levandowski, M.D.

Objectives:

The efficacy of influenza vaccine was evaluated according to the following studies:

1. The ability of influenza A/Beijing/353/89 (H3N2) vaccine strain, influenza A/Taiwan/1/86 vaccine strain and influenza B/Panama/45/90 vaccine strain to produce protective levels of cross reacting antibodies to new influenza A and B isolates in young and elderly age groups.

2. The ability of influenza B/Yamagata/16/88 vaccine strain to produce long lasting protective levels of antibodies in immunologically naive children as compared to antibodies induced by infection with related influenza strains, and to determine whether the immunologic priming is equivalent for the two means of immunologic priming.

3. The ability of repeated immunizations with the influenza virus vaccine to produce antibodies that are cross-reacting to relatively unrelated strains of the same subtypes of virus.

Methods Employed:

1. In the fall of 1991 trivalent subunit vaccine was used to immunize 57 healthy ambulatory elderly patients at Cornell University Medical Center in New York, 20 infirmed elderly at the Woodcrest Center in New Jersey, and 60 children and young adults at St. Vincent's Hospital in New York. The vaccine administered contained 15 micrograms of hemagglutinin for each of the strains included (A/Beijing/353/89, A/Taiwan/1/86, and B/Panama/45/90) and was administered by the intramuscular route. Blood samples were collected at the time of immunization and three weeks later. Antibody testing was done by means of hemagglutination inhibition (HI) in a microtiter system. Protective serum titers were presumed to be indicated by sera positive at dilutions 1:40 or

greater.

2. In the fall of 1991 trivalent subunit vaccine was used to immunize 36 children in Denver, Colorado. The children included a cohort of children who had never been immunized and had no history of infection by an influenza virus, a cohort who had been previously immunized , and a cohort who, by isolation of a virus similar to B/Panama/45/90 or by serologic result, were known to have been infected with an influenza B virus. The vaccine was administered as two doses of 7.5 micrograms or 15 micrograms of hemagglutinin for each of the strains (A/Beijing/353/89, A/Taiwan/1/86, or B/Panama/45/90). Blood samples were collected at the time of immunization and approximately three weeks later. Antibody testing for influenza viruses B/Panama/45/90 and B/Yamagata/16/88 were done by means of hemagglutination inhibition (HI) in a microtiter system using detergent disrupted purified viruses for antigen.

3. In the fall of 1991 trivalent vaccine was used to re-immunize 13 children who all had been immunized during the previous year. The vaccine in the first year had contained the hemagglutinins of influenza viruses A/Shanghai/16/89, A/Taiwan/1/86, and B/Yamagata/16/88; the vaccine in the second year contained the hemagglutinins of A/Beijing/353/89, A/Taiwan/1/86 and B/Panama/45/90. Blood samples were collected at the time of immunization and approximately three weeks later. Antibody testing for influenza B viruses (B/Panama/45/90, B/Yamagata/16/88, B/Victoria/2/87), and for influenza H1N1 viruses (A/Taiwan/1/86 and A/Chile/1/83) were done by means of HI in a microtiter system using detergent disrupted viruses for antigens.

Major Findings:

1. Among the healthy elderly, HI antibodies after immunization with the trivalent vaccine were present at dilutions of 1:40 or greater in approximately 67 % for A/Beijing/353/89, approximately 88 % for A/Taiwan/1/86, and approximately 80 % for B/Panama/45/90. Among the infirmed elderly, antibodies were present at 1:40 or greater in approximately 50 % for A/Beijing/353/89, in approximately 88 % for A/Taiwan/1/86, and in approximately 80 % for B/Panama/45/90. In the young, antibodies were present at 1:40 or greater in approximately 72 % for A/Beijing/353/89, in approximately 96 % for A/Taiwan/1/86, and in approximately 85 % for B/Panama/45/90. The geometric mean titers (GMT) of HI antibodies for the newly predominant H1N1 strain (A/Texas/36/91) was below the protective level and more than 2 times lower than for the vaccine strain among the elderly (mean age 83 years) and was below the protective level and more than 11 times lower than the vaccine strain among the young (mean age 17 years). The GMT for HI antibodies to newly identified influenza B strains gave varying results. For B/Guangdong/4/91 GMTs for the elderly and for the young were equal to or greater than the GMTs for the vaccine strain. However, for B/Quingdao/102/91 the GMT

was below the protective level and more than two times lower than the vaccine strain among the elderly, but was above the protective level and of similar titer to the vaccine strain for the young.

2. Among children immunized with a vaccine that included influenza B/Panama/45/90, HI antibody titers >1:32 for B/Panama/45/90 were found in the pre-immunization sera of none of the children not previously immunized or infected, 19% of the children previously immunized, and 83% of the children previously infected, and in the post-immunization sera of 78% of children never immunized or infected, 94% of children previously immunized, and 100% of children previously infected. The post-immunization GMT for each group also reflected previous immunologic exposures with the lowest titer in the children not previously immunized or infected (GMT=64) and the highest in the children previously infected (GMT=312). The post-immunization response of the children who had been previously immunized (GMT=91) was significantly less than that of the previously infected children. The HI titers using B/Yamagata/16/88 as antigen were not significantly different from those with B/Panama/45/90.

3. One of the children re-immunized had evidence of infection with an influenza B virus and two others had evidence of infection with an H1N1 influenza A virus as shown by rising antibody titers during the intervening influenza season. However, even including those children, the antibodies produced after re-immunization were not predominantly cross-reacting with strains of the same subtype that are unrelated to the vaccine or the infecting influenza virus strains. After re-immunization the GMT for B/Victoria/2/87 was <1:16 while the GMT for the vaccine strain (B/Panama/45/90) was 109. Similarly, the GMT for A/Chile/1/83 was 1:16 while the GMT for the vaccine strain was 79.

Significance to Program:

This contract continues to provide important information on the immunogenicity and efficacy of influenza vaccines, particularly with respect to the information needed to determine which influenza virus strains should be included in the commercially prepared vaccine. During the past year, the results obtained for serologic responses to influenza A H1N1 viruses in the United States were divergent with results obtained in Europe. The results from the serologic studies performed for this contract permitted completion of several supplementary investigations to determine the source of the divergent results, and were influential in the selection of the influenza A H1N1 vaccine component. The studies in infants continue to indicate that strategy for immunization specific for immunologically unprimed children may not yet be optimized.

Proposed Course of the Project:

Further elucidation of the causes of differences in immune responses among various age groups is planned and is beginning in studies already begun. The studies are particularly targeted to the very young and the very old in order to begin to identify the additional modifications to vaccine administration strategies can improve the use of existing and developing influenza virus vaccines.

Date Contract Initiated: August 14, 1980.

Current Annual Level: $413,000.00

Division of Virology
Center For Biologics Evaluation and Research

Progress Report
Contract No. 223-90-1107

Period: October,1 1991 through September 30, 1992.

Awarded to: Dr. William Burns, M.D. Johns Hopkins Oncology Center.

Project Officer: Hana Golding, Ph.D. Laboratory of DNA Virus Research, Division of Virology, CBER, FDA.

Title: Clinical study of HIV-1 and cytomegalovirus (CMV) infections in bone marrow transplant patients.

OBJECTIVES:

I. HUMAN CMV PROJECT:

I.A. To construct vaccinia vectors expressing either early genes (IE1,IE2), or late genes (envelope gB) derived from human CMV.

I.B. To test these vectors, after their introduction into EBV transformed autologous B cell lines, as targets for cytotoxic cells derived from CMV-seropositive individuals (potential bone marrow donors).

I.C. To correlate the levels of anti-CMV cytotoxic activity specific for either IE1/IE2 of gB in bone marrow recipients (or CMV seropositive AIDS patients) with the frequency and severity of CMV opportunistic infections.

I.D. The data generated in these studies will be applied in the design of CMV subunit vaccine aimed at generating protective anti-CMV cytotoxic T cells.

II. HIV-1 PROJECT:

II.A. To study the potential use of bone marrow transplantation in the treatment of AIDS patients with malignant lymphomas.

II.B. Recipients are treated with combination anti-viral therapies (AZT, DDI), followed by marrow ablative chemo-irradiation. Recipients are then infused with HLA compatible bone marrow cells and remain on anti-HIV-1 drugs to prevent infection of newly developing lymphoid cells.

II.C. Follow anti-HIV-1 cytotoxic cell responses (against GAG and ENV) prior to and following the bone marrow reconstitution of patients.

PROGRESS REPORT

I. HUMAN CMV

I.A. Vaccinia vectors expressing CMV IE1 and gB were successfully constructed and characterized by sequence analysis and monoclonal antibodies specific for IE1 and gB.

I.B. CBER received the above vectors, as well as a panel of HLA typed EBV-transformed B cell lines to be used as stimulators and targets in cytotoxicity assays.

I.C. PBL from future bone marrow donors and normal volunteers will be sent on a weekly base to the Division of Virology.

II. HIV1-1 PROJECT

II.A. Six patients were enrolled in the BMT + AZT protocol. Three with advanced lymphomas, and three AIDS patients with identical twins. The overall survival was low (1/6). Bone marrow engraftment was not delayed by the AZT treatment.

II.B. HIV-1 PCR analysis indicated that the hematopoietic reservoirs of HIV-1 in the recipients were significantly reduced (2-3 logs).

II.C. In two patients, HIV-1 was isolated from the BMT recipients and the isolated were found to be AZT resistant.

II.D. At the present time, there are no potential BMT recipients in the program.

II.E. Vaccinia vectors expressing GAG-POL and gp120 were obtained from Dr. Moss laboratory. In the absence of referrals, no CTL tests were conducted with them to date.

Date Contract Initiated:

September 1 1990

Current Annual level:

$ 286,000.00

LABORATORY SUMMARIES

LABORATORY OF DNA VIRUS RESEARCH

LABORATORY OF DNA VIRUS RESEARCH

October 1, 1991 through September 30, 1992
SUMMARY OF THE LABORATORY CHIEF

Unit on Molecular Biology and Biochemistry:
Head, Dr. Carol J. Marcus-Sekura

Dr. Marcus-Sekura is continuing a number of related projects designed to analyze immune responses to recombinant HIV proteins, and also to study the properties of the recombinant antigens. Several individual HIV-1 proteins have been expressed in *E. coli* vector systems. A project to express and study the viral integrase) (IN) (the virus protein required for integration of reverse transcribed viral DNA into the host cell genome) is continuing in collaboration with J. Levin (NICHD/NIH). In addition to characterization of DNA binding and enzymatic activity of HIV-1 IN, monoclonal antibodies to IN have been generated in collaboration with A. Karpas (Division of Bacterial Products/CBER) and Fab fragments from the monoclonal antibodies are being used in collaborative efforts with D. Davies (NIDDK/NIH) to obtain the three-dimensional structure of IN using X-ray crystallography.

Dr. Marcus-Sekura and Dr. Peng-fei Zhang who is a visiting scientist in her laboratory, is continuing a project to assess the potential of EBV gp350 as an EBV vaccine. gp350 is an EBV vaccine candidate since it contains the virus neutralizing epitope(s). MAbs to gp350 obtained from L. Qualtiere (University of Saskatoon) and G. Pearson (Georgetown Univ.) are being used to generate a physical map of the epitopes on the EBV membrane antigen gp350 in conjunction with recombinant forms of gp350 being generated in *E. coli* and Baculovirus vector systems, and to examine the role of glycosylation and conformation of the recombinant protein on the immune response.

Dr. Marcus-Sekura is continuing to attempt to answer regulatory questions posed by use of specific cell lines to produce biological products. Since many new recombinant biological products produced using the Baculovirus expression system use the insect cell line Sf9, and relatively little is known about the viruses which can infect Sf9 cells, Dr. Marcus-Sekura's laboratory is currently investigating whether any viruses pathogenic for humans can infect Sf9 cells. A series of viruses are being examined for their ability to infect Sf9 cells, and protocols for evaluating whether Sf9 cells are free of viral contamination and suitable for use for production of biological products are being developed.

Dr. Marcus-Sekura maintains her interest in mechanisms of antisense oligonucleotide inhibition of gene expression, although active research in this area has been curtailed due to time and personnel constraints.

Unit on Immunology: Head, Dr. Hana Golding

Dr. Golding is continuing several projects relating to

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

immunopathological aspects of HIV-1 infection and the anti-HIV therapeutic CD4. One project, performed in collaboration with G. Shearer (NCI/NIH) investigates the role of autoantibodies in the establishment of early immune dysfunction observed in many HIV infected patients. Sera from such patients were found to contain antibodies against a portion of HIV-1 gp41 which cross-react with a homologous region in human HLA class II antigens. The role of such antibodies in disease progression is being examined.

A second project in Dr. Golding's laboratory involves study of the role of CD4 in HIV entry into cells using mutant human T cells producing altered levels of CD4 and LFA-1 as well as reduced levels of DNA binding proteins of the NFkB family. Studies to correlate these deficiencies with HIV-1 susceptibility and reactivation by different cytokines are underway.

Another project in Dr. Golding's laboratory in collaboration with B. Golding (Division of Hematology/CBER) involves study of ways to immunize HIV-1 infected individuals whose immune system can provide only limited T cell helper function using HIV-1 virions or envelope-derived peptides conjugated to the T-cell Independent type 1 carrier (TI-1), inactivated Brucella abortus (or LPS derived from it). The study examines toe ability of the conjugates to generate long-term protective immunity (IgG) in normal and in T-cell deficient murine models.

Dr. Golding has recently begun a project in collaboration with D. Dimitrov and R. Blumenthal (NCI/NIH) to study HIV-1 env-mediated membrane fusion and syncytia formation using an assay based on the redistribution of fluorescent dyes between HIV-1 env-expressing cells and CD4+ targets. They are also examining the kinetics of sCD4 interactions with infected cells to further evaluate its potential use as an anti-HIV-1 therapeutic.

Unit on Oncology: Head, Dr. Abdur Razzaque

Dr. Razzaque and his group are conducting two projects relating to herpesviruses. One project being performed in collaboration with Clinton Jones (University of Iowa), seeks to identify and characterize transforming regions of human CMV. Current studies are exploring the effects of individual open reading frames in the mtrII region in transformation using recently constructed deletion clones. Dr. Razzaque is also involved in characterizing another transforming domain, mtrIII of CMV, and attempting to answer whether the DNA sequence or the ORF product is responsible for transformation.

A second project is to examine the oncogenic potential of the human herpesvirus, HHV-6, recently identified in AIDS patients and also in patients with lymphoproliferative disorders. Clones containing subgenomic portions of the virus genome are introduced into rodent (NIH 3T3) cells by DNA transfection, and the resulting cells are analyzed for morphologic transformation and production of tumors in nude mice. These studies also are being performed using human cells in collaboration with Dr. J. Rhim (NCI/NIH).

Dr. Razzaque is also working on a project in collaboration with Dr. R. K. Puri (Division of Cytokine Biology/CBER) to study metastatic potential and immunogenic properties of these tumors in mice.

Unit on Latency: Head, Dr. Philip Krause

Dr. Krause plans to continue work on HSV latency begun in collaboration with S. Strauss (NIAID/NIH). He is investigating the role, regulation, and mechanism of action of the HSV latency-associated transcripts (LATs) which are the only viral transcripts made while HSV is latent. Using clones (which he has constructed) containing the HSV-1 and HSV-2 LAT promoters, he is performing detailed comparisons of the promoters. To test the hypothesis that the HSV-2 LAT promoters are involved in reactivation from latency, a mutant HSV-2 virus unable to produce the LATs (which he has constructed) will be compared with wild-type and rescued viruses in animal models (in collaboration with L. Stanberry (Children's Hospital, Cincinnati). Using mutant clones (which he has constructed) in which the HSV-1 LAT promoter is substituted for the HSV-2 LAT promoter or the HSV-2 LAT promoter is substituted for the HSV-1 LAT promoter, he will attempt to generate the corresponding intertypic recombinant viruses. To test the hypothesis that differences in the LATs contribute to differing patterns of latency and reactivation between HSV-1 and HSV-2, these heterotypic viruses will be compared with wild-type and rescued viruses in animal models in collaboration with J. Pepose (Washington Univ., St. Louis) and L. Stanberry (Children's Hospital, Cincinnati).

Clinical Program on CMV

Ms. Manischewitz is monitoring patient specimens for effects of therapy in a series of clinical trials in collaboration with H. Masur and H.C, Lane (NIH Clinical Center) to study the occurrence of CMV infection in AIDS and the response of CMV infection in this setting to therapy. In collaboration with H. Masur, H.C. Lane, and M. Polis (NIH Clinical Center) and P. Smith (NIDR/NIH) she is also studying *in vitro* sensitivity of CMV clinical isolates to ganciclovir and foscarnet treatment.

Clinical Program on EBV

Mr. Armstrong is monitoring patient specimens for effects of vaccination with different HIV vaccines in clinical trials at the NIH in collaboration with J. Kovacs and H.C. Lane at the NIH Clinical Center to study the activation of EBV infection in EBV-positive HIV-negative vaccine recipients. In recent studies with lymphocytes from patients with EBV-associated aplastic anemia, he has isolated an IL2-dependent cell line secreting infectious HTLV-II. This is unusual since HTLV-II cell lines are normally transformed and IL2-independent, and studies to characterize the virus are in progress. Collaborative studies with N. Young (NHLBI/NIH) have been set up to screen additional aplastic patients for potential infection with HTLV-II.

LABORATORY OF RESPIRATORY VIRUS RESEARCH

Laboratory of Respiratory Virus Diseases

October 1, 1991 through September 30, 1992

Dr. Beeler expanded her studies into the antigenic composition of respiratory syncytial virus (RSV) F glycoprotein, considered the most essential part of the virus for infectivity and consequently most important to be neutralized by antibodies induced by a candidate vaccine. By developing an antigenic site blocking assay, she was able to analyze the antibody repetoire in human and cotton rats postinfectious sera. The assay holds promise in allowing to compare post-infection and post-vaccination antibody responses and how they correlate with protection against infection.

In a separate project Dr. Beeler used 14 RSV mutants she developed previously with mutations in the F glycoprotein as well as 2 prototype wild strains to infect susceptible cotton rats. She found that some of the mutants had reduced replication in the lower respiratory tract suggesting that changes in the F glycoprotein contribute to attenuation of RSV. Investigations continue along the same lines concerning the G glycoprotein to analyze its possible contribution to attenuation phenotypes of RSV.

Dr. Beeler's research is detailed in 3 separate reports titled "Antigenic and Molecular Characterization of the Fusion Glycoprotein of RSV," "Sequencing of PCR Amplified cDNA of the Gene for the RSV Fusion Glycoprotein," and "Immune Response to Formalin Treated Respiratory Syncytial Virus."

Dr. Levandowski continued his research on human rhinoviruses (HRV) and on influenza immunization.

Of 53 guinea pig sera produced against 53 different serotypes, only homologous sera (tested with HRV 2 and HRV 14) had neutralizing activity. However the majority of the additional 51 sera, although not neutralizing, reacted with the VP1 component of HRV 2 and HRV 14. The pattern of reactivity correlated best with the known spectrum of HRV susceptibility to antiviral substances which stabilize the rhinovirus capsid by binding to a relatively conserved amino acid sequence on the surface. The data suggest that common antigenic determinants conserved in the evolution of HRV may be important reactive sites of the viruses.

In the area of immunity to influenza infection a study was performed in which toddlers or very young children primed with natural or inactivated influenza B virus were reimmunized one year later together with a susceptible control cohort using inactivated influenza B vaccine. There was a significant difference in the post-vaccination GMT which rose from 48 to 312 in the previously infected children, from <16 to 91 in the previously vaccinated group and from <16 to 64 in the previously unimmunized/uninfected group. The results suggest that a separate strategy of immunization (live vaccine--> killed vaccine) would be advantageous for young children and infants.

In a separate project Dr. Levandowski developed are PCR assay capable of detecting contamination of recombinant seed virus by components of the parental virus used in the production of the recombinant. The PCR technique is approximately 100-fold more sensitive than serological techniques available so far.

Dr. Levandowski's research is detailed in three separate reports titled, "Cellular and Humoral Immune Responses to Rhinovirus," Influenza Vaccine Development and Use," and "Influenza Reassortant Virus: Biology and Genetics."

LABORATORY OF PEDIATRIC DISEASES

Laboratory of Pediatric Diseases

October 1, 1991 through September 30, 1992

Several projects in the Laboratory dealt with improving techniques to measure the potency of Inactivated Poliovirus Vaccine (IPV) and were spearheaded by Dr. Leigh Sawyer.

One project compared the performance of reagents for the ELISA potency test produced by three National Control Authorities and two manufacturers. Capture polyclonal sera were used in conjunction with monoclonal or polyclonal detection antibodies derived from four different animal species. An outgrowth of this project is the organization of an International Collaborative study under the auspices of WHO for standardizing the ELISA test for IPV potency and measurement. Collaborators received an ELISA kit containing plates, monoclonal and polyclonal antibodies and monotypic antigens. The study is in progress. Seven manufacturers and six national control authorities participate in the study.

Another project investigated the effect of thimerosal, a common preservative, on the antigenicity and immunogenicity of IPV, a situation which might arise in combination vaccines. It was found that the antigenicity of type 1 and type 2 antigen and less of type 3 antigen as measured by the ELISA test was reduced by thimerosal over a 2 week period. This reduction however did not correlate with reduced immunogenicity in mice. These findings have important implications where considering preservatives in combination vaccines.

Developing an animal model to measure the in vivo immunogenicity of IPV is the last project of Dr. Sawyer. Tests using rats, guinea pigs or chickens were found cumbersome or unsatisfactory. Tests in Balb C mice show that there is a good correlation between a dose of trivalent vaccine injected and neutralizing antibody response. Correlation between the mouse test and human immunogenicity will be the next step in this project.

Dr. Albrecht in collaboration with CDC investigated the serological response of US children to two measles virus vaccines, one US-licensed and the other an experimental vaccine (Edmonston-Zagreb) found in studies abroad to be highly immunogenic in infants

in the presence of maternal antibodies. The experimental vaccine did not appear to be more immunogenic than the licensed vaccine in children 9 months of age whether they did or did not have circulating maternal antibodies. The experimental vaccine was more immunogenic when used in a tenfold higher concentration, a dosage not recommended for routine use, however.

In a separate study, an assessment was made of measles antibody titers in mothers borne before 1957 who are likely to have been immunized by wild virus infection and in young mothers likely to have measles vaccine-induced immunity. The GMT in sera of older mothers was 4707 (n=913) as opposed to a GMT of 1592 in younger mothers (n=3861; p<0.001). Children born to younger mothers were more likely to be seronegative than those born to older mothers at 6 months and 9 months, but not significantly at 12 months of age. Nevertheless, the response to measles vaccine at 12 months of age was higher in children born to younger mothers than compared with older mothers (94% vs 83%, NS).

Dr. Lundquist investigated conditions under which live poliovirus vaccine can be lyophilized to improve conditions for its storage in the cold chain and facilitate immunization programs. Under standard conditions of lyophilization the virus titer dropped by 4.0 log10. Adding of salts, organic solvents, detergents or changes in pH alone or in combination achieved a 1-2% survival of original infectivity at best. A significant improvement was achieved with a lipid based reagent preserving 30-40% of viral infectivity on lyophilization.

Another project of Dr. Lundquist looked at an in vitro polio translation system based on Hela cell extracts. The characteristics of the response to wild type viral RNA are being determined and will be used in comparison with transcription of attenuated virus RNA for an understanding of the mechanisms in viral attenuation.

Dr. Jackie Muller collaborated with a number of laboratories requiring expert electron microscopy evaluation. One project concerned the identification of macrophage-like cells in the peritoneal fluid of mice with MAIDS syndrome.

In another project the cell morphology of infection with standard Sindai virus and standard virus spiked with defective Sundai particles was evaluated. The former cells appeared relatively healthy in spite of a heavy viral antigen burden, while Sundai and DI particle infected cells showed early disintegration with little evidence of nucleocapsid synthesis.

Liver biopsies of chimpanzees experimentally infected with hepatitis C were examined for morphological evidence of infection.

The Laboratory of Pediatric Diseases provided short-term laboratory training in vaccine potency testing and viral antibody measurement to the following individuals:

Dr. Sam Ratnam - Newfoundland - Newfoundland Public Health
8-6-92 to 8-8-92 Laboratory

Dr. Scott Krugman - New York - New York University
7-13-92 to 7-14-92

Dr. M. Xueref - France - Pasteur-Merieux
5-19-92 to 5-21-92

Ms. Evanilce Ferreira - Rio de Janiero, Brazil
9-9-91 to 10-3-91

Dr. Jorge F. Gomez Herrera - Mexico City, Mexico
C. Pedro Garcia Banuelos Gerencia General de Biologics
January 1992 Y Reactivos

LABORATORY OF RETROVIRUS RESEARCH

Laboratory of Retrovirus Research

October 1, 1991 through September 30, 1992

Dr. Klinman's research focused on three areas related to HIV infection. The first concerned the humoral immune response to HIV-1 envelope proteins. Glycosylated recombinant gp120 was found to act as a more efficient immunogen than non-glycosylated envelope glycoprotein or the V3 loop region specific peptides.

A transgenic murine model of HIV expressing gp120 protein was examined. As expected, animals were tolerant to gp120 but not other HIV-1 proteins. SCID mice were reconstituted with PBLs from gp160 vaccinated human volunteers. HIV-1 reactive PBLs were present initially in the peripheral circulation and then homed to the bone marrow of immunized individuals.

The pathogenesis of polyclonal activation and (auto) antigen specific immune stimulation was closely investigated in a mouse SLE model. Results indicated that the two processes combine to induce the humoral abnormalities found in disease states.

Dr. Arifa Khan studied the genetic relationship among primate lentiviruses especially HIV-1, HIV-2 and SIV. Nested PCR analysis was performed on DNAs from African and Asian monkeys and humans with probes based on the pol region of SIV or HIVs. Amplified fragments were obtained from all species and currently cloned to determine evolutionary relatedness of these endogenous sequences.

Her other project was aimed towards developing a primate HIV model using pig-tail (macaque) monkeys. The in vitro sensitivity of PBLs to low and high passage HIV-1 isolates was investigated. The goal is to vaccinate and show protection against the prevalent MN strain of HIV-1.

Dr. Quinnan in collaboration with Ms. Luba Vujcic and Ms. Wendy Glass researched three major projects.

Up to three sequential HIV-1 isolates were obtained from 5 patients over a 2.5 to 5 year period and tested against the patient's sera. With time isolates became more readily neutralizable. The isolates will be studied by PCR if corresponding changes can be identified in or outside the V3 loop region.

Reference sera with neutralizing titers to HIV-1 were prepared for use on an international basis sponsored by WHO. A collaborative study was conducted with six laboratories in Europe and the United States participating. Mean titers were calculated for each of the sera specific for the HIV_{MN}, HIV_{IIIB}, HIV_{RF} and HIV_{SF2} strain, respectively. A value in international units of antibody will be assigned before the samples are made available to WHO.

Synthetic peptides of the V3 loop region were prepared and used as blocking agents in a neutralization assay against HIV-1 isolates from the United States, Zaire and Brazil. Peptides representative of the MN strain blocked most effectively. Geographic prevalence of strains based on the results of blocking assays is being evaluated.

LABORATORY OF HEPATITIS RESEARCH

ANNUAL REPORT

Laboratory of Hepatitis Research

October 1, 1991 through September 30, 1992

The Laboratory of Hepatitis Research has been involved in 2 major research projects involving hepatitis A virus (HAV) and hepatitis C virus (HCV). The HAV project has been directed towad identification and characterization of the cellular receptor for HAV. This project has many important implications. The receptor may be related to organ tropism and species specificity. At present HAV is thought to replicate almost entirely in hepatocytes and exclusively in primates. Beside providing basic information on viral replication and pathogenesis, identification of the receptor may permit the development of a small animal model for HAV which would greatly facilitate HAV research in areas such as pathogenesis and vaccinology. It may eventually prove useful for evaluating licensed vaccines for safety and potency. In related experiments, the HAV genome is being modified in an effort to improve its *in vitro* replication and immunogenicity *in vivo*.

We are studying the pathogenesis of HAV infections in the chimpanzee model by selecting specific T-cell clones and analyzing the epitopes to which they are directed. Using the "Pepscan" system we are looking at large numbers of oligopeptides in an effort to find these specific epitopes. T-cell eptopes may have important implications for synthetic vaccines.

We are evaluating a potential synthetic vaccine developed by Stapleton at the University of Iowa. He has inserted the entire open reading frame of the HAV genome into a vaccinia virus expression system. The vaccinia not only produce HAV specific protein, but the polyprotein is processed and there seems to be particle assembly. This may make an ideal vaccine that has the potential to overcome some of the problems with tissue culture produced vaccines.

HCV research has been broad ranging. We have constructed full length clones of the HCV genome and are beginning a process to evaluate them for infectivity. If these clones are infectious it will permit genetic manipulation of HCV that may result in a virus with the necessary characteristics for a vaccine such as growth in cell culture or loss of the ability to induce chronic infections in man. We are characterizing the antigenic structure of HCV for both B-cell and T-cell epitopes. Using human monoclonal antibodies derived from chronically infected individuals, we have found a

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previously unrecognized epitope near the C-terminus of the core protein. We are presently evaluating human monoclonal antibodies to the envelope glycoproteins. In collaboration with H. Greenberg and H. Hsu at Stanford University we are making a series of mouse monoclonal antibodies to the structural proteins of HCV. We have expressed the structural proteins by means of recombinant baculoviruses and have used these expressed proteins for analysis of the various monoclonal antibodies. In addition these expressed proteins have been useful as diagnostic reagents. Amongst chronically infected patients, we find as expected 100% have antibody to the core protein, but surprisingly between 15 and 30% also have antibody to one or both of the surface glycoproteins.

We are studying the role of T-cells in HCV infections from the point of view of both immunity and pathogenesis. In collaboration with Dr. J. Berzofski, NCI we have identified a cytotoxic T-cell epitope in the NS5 (polymerase) protein of HCV using mice immunized with a recombinant vaccinia virus. We have also selected T-cell lines from both chronically infected patients and experimentally infected chimpanzees. These line are being evaluated for cytotoxic and helper activity.

In collaboration with Charles Rice of Washington University, we have been studying the HCV protease (NS3) and the processing of the HCV polyprotein. We have expressed the protease in a bacterial system and identified the enzymatic active site. We have also identified the cleavages that are performed by the viral protease and which are performed by host enzymes. We hope to purify the enzyme and eventually evaluate its 3 dimensional structure. We have also shown that the protease NS3 does not require the presence of the NS2 protein as it does for yellow fever virus and dengue virus.

Regulatory work by members of this lab includes monitoring of potency of licensed hepatitis B vaccines and lot release of hepatitis B vaccines. Membership on PLA committes for the licensing of interferon for chronic hepatitis B, diagnostic tests for hepatitis C, licensing of a Japanese encephalitis virus vaccine and hepatitis A vaccine. Numerous IND reviews for a broad variety of products have been performed.

LABORATORY OF VECTOR-BORNE VIRAL DISEASES

Annual Report

Laboratory of Vector-borne Virus Diseases

Period: October 1, 1991 through September 30, 1992

The laboratory of Vector-borne Virus Diseases (LVVD) officially came into being in the spring of 1992. Two of the members of LVVD, Lab Chief Lewis Markoff, M.D., and Microbiologist Christine Winter had transferred to FDA from NIH in August, 1991, as adjunct members of the Laboratory of Pediatric Diseases. An additional two members of LVVD, Senior Staff Fellow Barry Falgout, Ph.D., and Microbiologist Stephanie Polo, were hired in January, 1992. A fifth member of LVVD, Audrey Chang, Ph.D., commenced work as an NRC Fellow on July 1, 1992.

LVVD is nominally charged with the performance of FDA-mandated regulatory duties for biological products relating to viruses and the conduct of research on epidemiologically important vector-borne viruses. These include members of the families bunyaviridae, arenaviridae, togaviridae, and flaviviridae. The research effort in LVVD is currently confined to viruses of the family flaviviridae, especially the dengue (DEN) viruses, Japanese encephalitis virus (JEV), and in the future will include hepatitis C virus (HCV). All three of these agents cause disease of major importance. DEN viruses (four serotypes) are endemic in tropical and sub-tropical areas supporting propagation of the vector Aedes mosquito. For example, the disease dengue fever and its complications were the leading cause of pediatric hospitalization in Thailand in 1987. Although dengue fever is not common in the U.S., Aedes mosquitoes are found in the southern states, and an epidemic of dengue fever is said to have occurred in Philadelphia 200 years ago. No satisfactory vaccine is available. JEV is a major cause of illness in southern China and caused significant disease in rural areas of Japan in both humans and pigs prior to the widespread use of a killed virus vaccine in that country. This vaccine is currently under consideration for licensure in the U.S. An attenuated strain of JEV is

in use experimentally as a vaccine in China. HCV is the recently discovered major cause of post-transfusion ("Non-A, Non-B") hepatitis. Although not known to be vector-borne, HCV most resembles flaviviruses and pestiviruses (members of the family togaviridae) at the molecular level. Much effort is currently being expended on the development of a vaccine against this agent and on techniques for rapid diagnosis of infection.

A major thrust of our research effort on the dengue viruses is in the area of vaccine development. It has long been known that genomic RNA isolated from positive-stranded viruses is potentially infectious when transfected into susceptible cells. This was originally demonstrated for the picornavirus, poliovirus, and has been demonstrated for a number of flaviviruses. Recently, full-length cDNA clones of yellow fever virus and of DEN type 4 virus (DEN4) have been constructed, and RNA transcripts synthesized in vitro from them have also been shown to be infectious. This capability provides a basis for creating cloned attenuated flaviviruses by site-directed mutagenesis of cDNA, if full-length cDNA bearing a known set of mutations is used as the template for in vitro synthesis of infectious RNA transcripts. Mutant viruses derived in this manner can be used to elucidate mechanisms of virus replication and to study pathogenesis. Viruses which appear suitably attenuated for virulence in animals may be vaccine candidates. Currently, we are attempting to assemble a full-length cDNA copy of the genome of DEN2 in order to commence with these studies. A mouse-adapted strain of DEN2 was chosen, because it is more reliably virulent in mice (causing fatal encephalitis after intracerebral inoculation) than is DEN4, from which the currently available full-length cDNA was constructed. Other current research projects in LVVD, designed to investigate the nuclear localization and ubiquitination of the capsid protein, processing of the viral structural proteins pre-membrane (prM) and envelope (E), the fine specificity of the association of the virally encoded protease, NS3, with its cooperative ligand, NS2B, and the mechanism of initiation of RNA transcription, are intended in part to help define regions of the flavivirus genome in which non-lethal mutations could be introduced for the purpose of generating attenuated mutant

viruses.

A second thrust of our work on flaviviruses is toward identification of their cellular receptors. It has recently been shown that DEN viruses infect cultured human skin cells resulting in the production and release of cytokines which may play a role in disease pathogenesis. Since it is possible that virus binding to receptors mediating cytokine release is the direct cause of the stimulation that was observed, and since it is likely that infection of skin cells is the initial event in mosquito-borne flavivirus infection, we have decided to direct our initial effort at identification of DEN virus receptors on this substrate. Currently the problem is being addressed in two ways. The first involves use of the "Virus Overlay Protein Binding Assay" (VOPBA). In this assay, membranes are prepared from the desired cell line, solubilized, and separated on polyacrylamide gels under denaturing or nondenaturing conditions. Proteins are then blotted onto a membrane and exposed to virus which is detectable either by radiolabel or by antibody. Thus virus-binding membrane proteins can be identified. The second approach involves the preparation of monoclonal antibodies against cell surface proteins in the hope of finding a monoclonal antibody that binds the virus receptor, as indicated by its ability to block infection of the cells under study.

In the future, we plan to study other virus-cell interactions. (1) Mosquito-borne flaviviruses like the DEN viruses and JEV show marked species specificity for their mosquito vector hosts. Available evidence suggests that this specificity is a product of the infectability of the midgut epithelium of the mosquito by a particular flavavirus, which could be mediated at the level of the cellular receptor. Identification of virus receptors in different mosquito species could verify this hypothesis. If mosquito cell receptors are identified, prevention of transmission by anti-mosquito-receptor antibodies may prove a feasible method for controlling the spread of disease in endemic areas. (2) Pathogenic flaviviruses can be divided into two groups comprising those that do cause a meningo-encephalitis and those that do not. Antigenic analyses of flavivirus species, using antisera that primarily recognize E, the major surface

antigen of the virus, reveals that encephalitis-causing flaviviruses are antigenically similar. Since E appears to be the viral attachment protein, these data are consistent with the possibility that antigenic similarity of E's of encephalitis-causing flaviviruses could be related to adaptation to attachment and growth in neuronal cells. Identification of JEV receptors on neuronal cells could help to elucidate this important pathogenetic difference among flaviviruses.

INTRAMURAL RESEARCH PROJECTS

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01001-08 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epitope Mapping of the EBV Membrane Antigen

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.J. Marcus-Sekura, Ph.D. Chief, LDNAVR, DV, CBER
P.F. Zhang, Ph.D., LDNAVR, DV, CBER
M. Klutch, Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

providing MAbs: L. Qualtiere, Univ. Saskatchewan, Saskatchewan, Canada and
G. Pearson, Georgetown Univ., Washington , DC

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The EBV membrane antigen (EBV-MA) gp350 contains virus neutralizing epitopes and is currently being tested as a possible EBV vaccine candidate in England. A series of 15 monoclonal antibodies (mAbs) to EBV-MA were obtained from Louis Qualtiere and Gary Pearson. These have been used to screen antigen expressing clones for reactivity to different epitopes in order to identify the nucleic acid sequences important for virus neutralization. Initially ten overlapping clones which express different portions of unglycosylated gp350 were constructed in E. coli. Four of fifteen mAbs tested reacted with the recombinant E. coli antigens, and three antigenic epitopes (nucleotides 1980-2307, 3186-3528 and 3528-3576 in Bam HI L) identified. Proteins expressed by the clones were used to produce antisera in rabbits. When tested for neutralization of EBV in tissue culture, the rabbit antisera as well as the mAbs which recognized the three epitopes failed to neutralize, suggesting that these epitopes are not involved in neutralization. A manuscript describing these data has been published in J. Gen. Virol. Since the remaining mAbs may only recognize the glycosylated form of gp350, the entire gp350 coding sequence has also been expressed in a Baculovirus expression system which produces glycosylated protein. All of the MAbs react with the baculovirus expressed protein. Immunofluorescence studies of Sf9 cells expressing gp350 suggest the protein is membrane associated. Five additional subclones which express different portions of gp350 in the Baculovirus expression system have been prepared and used for additional epitope mapping studies. Recent data indicate that these subgenomic proteins do not react with all the mAbs recognized by the entire Baculovirus-expressed gp350.

PHS 6040 (Rev. 5/92)

Publications related to project Z01 BF 01001-08 LDVR

Zhang PF, Klutch M, Armstrong G, Qualtiere L, Pearson G, Marcus-Sekura CJ. Epitope mapping of EBV gp350 using monoclonal antibodies and recombinant proteins expressed in E.coli defines three antigenic determinants, J Gen Virol 1991; 72:2747-2755.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01002-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Expression of HIV Antigens in E. coli

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.J. Marcus-Sekura, Ph.D. Chief, LDNAVR, DV, CBER
A.M. Woerner, MS, Microbiologist, LDNAVR, DV, CBER
M. Klutch, Microbiologist, LDNAVR, DV, CBER
J.K. Smith, Chemist, LDVR, DV, CBER

COOPERATING UNITS *(if any)*

On aspects of the project involving: a) rabbit antisera - Dr. J. Levin, NICHD, NIH
b) crystallization of IN using IN MAb - Dr. D. Davies, LMB, NIDDK, NIH
c) production of ascites - Dr. A. Karpas, LAI, DBP, CBER

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

INSTITUTE AND LOCATION

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.5	0.7	1.8

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

HIV integrase (IN) is a viral enzyme required for integration of virus DNA into the host chromosome. This type of integration is highly specific for retroviruses, and HIV-1 IN is therefore a target for antiviral therapy. To design antivirals, it is essential to characterize IN. We have expressed the IN gene in E. coli as a fusion protein. The cloned IN is reactive with both HIV-1 and HIV-2 positive patient sera in ELISA, while rabbit antisera to the recombinant protein are reactive only with HIV-1 IN but not HIV-2 IN by Western blot. These data have been published in AIDS Res. Hum. Retro. Hybridomas have been prepared using the IN expressing clone and the MAbs are reactive with HIV-1 but not HIV-2 by Western blot. An invention report has been filed and a manuscript is in preparation. Additional clones expressing the N- and C-terminal halves of IN have been constructed and the proteins they express examined for ability to bind DNA using a Southwestern blotting procedure. The complete IN molecule as well as the C-terminal protein bind DNA; the N-terminal portion exhibits no binding activity, suggesting that the C-terminal region contains the DNA binding site. A manuscript has recently been published in AIDS Res. Hum. Retro. We have purified our full-length recombinant IN and it exhibits activity in a specific *in vitro* assay for enzyme activity involving cleavage of 2 bp from an oligonucleotide corresponding to the HIV-1 LTR. Since our IN is produced as a fusion protein containing 13 amino acids of lambda cII at its amino terminus, detection of activity with our protein indicates that a free amino-terminus is not required for activity. This may be relevant to the in vivo situation, since IN is initially synthesized as a polyprotein linked to RT at its amino-terminus.
To more precisely localize the DNA binding site within the C-terminal half of the molecule, we have constructed a series of additional subclones and we are currently purifying the deleted proteins they produce for analysis of DNA binding. We have recently established a collaboration with David Davies at the NIH to provide him with large quantities of IN MAb for generation of Fab fragments for X-ray crystallography of IN, which may eventually facilitate design of enzyme inhibitors.

Publications related to project Z01 BF 01002-09 LDVR

Woerner AM, Klutch M, Levin JG, Marcus-Sekura CJ. Localization of DNA binding activity of HIV-1 integrase to the C-terminal half of the protein, AIDS Res Hum Retroviruses 1992; 8:303-310.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01003-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Inhibition of Gene Expression by Normal & Modified Antisense Oligonucleotides

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.J. Marcus-Sekura, Ph.D. Chief, LDNAVR, DV, CBER
M. Klutch, Microbiologist, LDNAVR, DV, CBER
J.K. Smith, Chemist, LDVR, DV, CBER

COOPERATING UNITS *(if any)*

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TOTAL STAFF YEARS: 0.2	PROFESSIONAL: 0.1	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project was initially undertaken in order to determine whether modified oligonucleotides of appropriate antisense sequence can inhibit biological reactions, and to examine their potential as antiviral agents. A transient expression system in eukaryotic cells was used to see whether normal and modified oligonucleotides could inhibit the activity of the enzyme chloramphenicol transferase CAT (the gene for CAT was introduced into the cells by DNA transfection). It was determined that micromolar concentrations of modified oligonucleotides could inhibit CAT activity and that chain length, concentration, and nature of the chemical modification of the oligonucleotide could influence the degree of inhibition. This work has been published (1987) as well as a second paper discussing techniques for using antisense oligonucleotides has been published (1988). Experiments using antisense oligonucleotides have now been extended to study potential antiviral effects using EBV gene expression as a model. Antisense molecules appear to inhibit expression of a specific EBV early antigen, EA(D), induced by stimulation of B95-8 cells with the phorbol ester TPA as assayed by immunofluorescence and ELISA using a monoclonal antibody to EA(D). Additional antisense sequences are being examined to determine the specificity of this inhibition. We have purchased an instrument to synthesize oligonucleotides for these experiments and J. Smith has recently joined the laboratory, which should help greatly to supply materials which have been a limiting factor in this project. We have developed protocols for large-scale synthesis of antisense oligonucleotides and purification by HPLC, and implementation is planned in the near future. Temporarily, active research in this area has been curtailed due to time and personnel constraints.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01004-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection of CMV infections in AIDS patients in clinical protocols

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.F. Manischewitz, Microbiologist, LDNAVR, DV, CBER
H. Golding, Ph.D., Supervisory Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

H.C. Lane, M.D., Chief, CMRS, LIR, NIAID, NIH
H. Masur, M.D., Director, CCM, CC, NIH

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Laboratory of DNA Virus Research

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since CMV is recognized as one of the leading opportunistic infections in AIDS patients, it is important to monitor blood, urine, and tissue specimens from patients enrolled in various clinical trials for the presence of CMV. The following clinical protocols are currently in progress:

(1) Combination therapies against HIV-1 : AZT/IL2, IFN-alpha /IL2, and a three arm trial of combination AZT, IFN, and AZT/IFN. In addition, we are following a small group of identical twins (only one is HIV-1 infected) who have undergone autologous bone marrow transplantation.

(2) Another goal of this project is to determine if CMV can be isolated from bronchial lavage specimens taken from asymptomatic as well as symptomatic HIV-1 patients. It may provide an early indicator of reactivated CMV in these patients, and lead to early initiation of anti-CMV treatment to prevent systemic fulminating disease.

(3) Clinical trial with the drug foscarnet (trisodium phosphonoformate) for the treatment of HIV-1 patients with CMV retinitis. Improvement was documented by a reduction of CMV isolation from tissue specimens, improved vision, and reduced HIV-1 p24 levels.

(4) A randomized, double blind, controlled trial to determine the efficacy of CMV-immuneglobulins for prophylaxis of CMV infections in AIDS patients.

PHS 6040 (Rev. 5/92)

Publications related to project Z01 BF 01004-10 LDVR:

Palestine AG, Polis MA, De Smet MD, Baird BF, Falloon J, Kovacs JA, Davey RT, Zurlo JJ, Zunich KM, Davis M, Hubbard L, Brothers R, Ferris FL, Chew E, Davis JL, Rubin BI, Mellow SD, Metcalf JA, Manischewitz J, Minor JR, Nussenblatt RB, Masur H, Lane HC. A randomized, controlled trial of Foscarnet in the treatment of cytomegalovirus retinitis in patients with AIDS, Ann Intern Med 1991; 115:665-673.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01006-05 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Chemically induced T cell mutants resistant to HIV-1 infection

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. Golding Ph.D. Supervisory Microbiologist, LDVR, DV, CBER
J. Qian M.D. Ph.D. Staff Fellow, LDVR, DV, CBER
J. Manischewitz Microbiologist, LDVR, DV, CBER

COOPERATING UNITS *(if any)*

U. Siebenlist Ph.D., NIAID
M. Norcross M.D., DCB, CBER

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.8	1.4	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A panel of mutants were derived from the human T cell line clone CEM, expressing different levels of surface CD4 receptors and demonstrating different susceptibilities to HIV-1 infection.
Multiple assays were developed to analyze these clones: (a) Quantitative flow cytometry analysis of surface receptors and binding of soluble gp120. (b) Quantitative PCR to measure viral entry and proviral DNA formation. (c) Vaccinia-gp160-vector based syncytia assay. (d) Gel retardation assays to study the levels of DNA binding proteins required for optimal HIV-1 proviral reactivation. (e) Protein Kinase C enzymatic activity assay. (f) Chloramphenicol acetyl transferase (CAT) assay to measure activation of the HIV-1 LTR.

Major Findings:
I. No direct correlation exist between the number of surface CD4 receptors and the kinetics of HIV-1 infection. Subclones expressing less than 1000 CD4 receptors/cell were equally susceptible to HIV-1 lab strains as the parental CEM line.
II. Two CEM subclones were isolated with dramatically reduced susceptibility to infection by multiple strains of HIV-1. These mutants were found to have a markedly reduced level of DNA binding protein belonging to the NFk-B family (p50). Experiments are under way to establish the underlying mechanisms for this deficiency, and to reconstitute the mutants by expression vectors containing wild type p50.
The knowledge gathered in this project will be applied for the studies of neonatal T cell infection. Studies will correlate the kinetics of HIV-1 infection with induction of DNA-binding proteins, lymphokine production, and activation state.

Publications related to project Z01-BF 01006-05

Gruber MF, Hewlett IK, Sims T, Vujcic L, Manischewitz J, Golding H. Study of viral entry and replication in HIV-1 infected CEM T cell subclones which are reduced in their ability to form syncytia, AIDS Res Hum Retroviruses; 1992; 8:729-736.

Hillman K, Qian J, Siegel JN, Roderiquez G, Blackburn R, Manischewitz J, Norcross M, Golding H. Reduced susceptibility to HIV-1 infection of EMS-induced CEM mutants correlates with a blockade in their protein kinase C signaling pathway, J. Immunol. 1992; 148:3991-3998.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01007-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

HIV-1 gp41-induced immunosuppressive crossreactive autoantibodies.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. Golding Ph.D. Supervisory Microbiologist, LDNAVR, DV, CBER
R. Blackburn, Biologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

G. Shearer Ph.D. Senior Investigator Exp. Immunol. Branch NCI, NIH.
D. Mann M.D. Viral Carcinogenesis Branch, NCI, NIH
Prof. Hermann Katinger M.D. Institute of Applied Microbiol. Vienna, Austria.

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Laboratory of DNA Virus Research

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.0	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We reported the identification of highly conserved, homologous regions located in the carboxy-terminus of HIV-1 gp41 (a.a. 837-844), and the N-terminal of human HLA class II beta chains (DR,DQ, a.a. 19-25). This "molecular mimicry" led to the generation of anti-gp41 antibodies which crossreact on "native" class II molecules (CRab). The class II binding activity was demonstrated using : (a) Murine anti-gp41-peptide monoclonal antibodies (mAb). (b) Human mAb 4E10 derived from asymptomatic HIV-1 infected patient. (c) 30-40 % of HIV-1 infected individuals in either early stages (WR 1-3) or late stages of AIDS (WR 4-6). In biological assays it was found that the CRab could block normal immune responses to recall antigens such as tetanus toxoid and flu. These antibodies could also mediate antibody dependent cellular cytotoxicity of HLA class II-positive cells (e.g. B cells, macrophages, activated T cells).

In asymptomatic patients (CD4 numbers > 500/ml), production of CRab correlated ($p<0.001$) with early loss of T cell responses to recall antigens as measured by proliferative responses and IL2 production in vitro.

In a cohort of HIV-1 infected hemophiliacs, high titers of CRab was found to correlate with rapid progression (2-4 yrs) to full blown AIDS.

We are currently continuing to monitor in parallel : (a) HLA typing, (b) Disease progression, and (c) CRab production, in a cohort of patients from whom multiple sera samples and clinical data are available.

We also test sera from various vaccine groups who were immunized with subunit vaccines containing the gp41/HLA class II homologous region, for the presence of CRab.

Publications related to project Z01 BF 01007-05

Blackburn R, Clerici M, Mann D, Lucey DR, Goedert J, Golding B, Shearer G, Golding H. Common sequence in HIV-1 gp41 and HLA class II beta chains can generate crossreactive autoantibodies with immunosuppressive potential early in the course of HIV infection. In: Atassi MZ, ed. Advances in immunobiology of proteins and peptides VI. New York: Plenum Press 1991; 63-69.

Golding H, Blackburn R, Steindl F, Shearer GM, Mann D, McCutchan F, Mascola J, Katinger H, Golding B. Autoimmune response in HIV-1 infection. In: Levy J, Weiss R, Neu HC, eds. Frontiers of Infectious Diseases: Focus on HIV. London: Churchill Livingstone, 1992; (n press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01008-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Production of anti-HIV-1 T-independent vaccine.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. Golding, Ph.D., Supervisory Microbiologist, LDNAVR, DV, CBER
M. Zaitseva, Ph.D., Fogerty Fellow, LDNAVR, DV, CBER
J. Qian, M.D. Ph.D., Staff Fellow LDNAVR, DV, CBER
J. Manischewitz, Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

B. Golding, M.D. Medical Officer LCB, DH, CBER
M. Betts, Biologist LCB, DH, CBER

LAB/BRANCH

Laboratory of DNA Research

SECTION

Immunology

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In HIV-1 infected individuals, CD4-bearing T cells become functionally deficient early in the course of infection, and are numerically depleted in advanced stages of the disease. Also, it was found that the ratio of TH1 to TH2 undergoes reversion in many patients who show signs of deterioration. In designing new subunit vaccines for immunization of high risk individuals or for boosting seropositive individuals, four goals were set: (a) to identify HIV-1 subunits which will generate widely cross neutralizing antibodies, (b) to identify a carrier which will increase the immunogenicity of the HIV-1 subunits, (c) to identify a carrier which will recall anti-HIV B memory cells in patients with pre-existing immune deficiency, and (d) to identify a carrier which would augment the TH1/TH2 ratio of infected individuals. The gram negative Brucella abortus (Ba), and LPS derived from its cell wall (Ba-LPS), were found to fulfill the last three goals. Inactivated HIV-1 linked to Ba, was found to be more immunogenic than unconjugated HIV-1, not only in normal mice, but also in CD4-depleted mice. The antibodies generated were predominantly of the IgG2B/2A, and they blocked efficiently HIV-1 env-mediated syncytia formation.
In recent studies it was found that the Ba or its LPS are much less toxic to animals than E. Coli derived LPS. More importantly, it was found that Ba and Ba-LPS activate directly purified human CD4-positive TH1 cells, and to a lesser degree, CD8-positive cells, as judged by induction of the lymphokines IL2 and IFN-gamma. We are currently employing sensitive PCR techniques to measure lymphokine production by adult and neonatal T cells activated by Ba. We also studying what DNA-binding proteins are induced following Ba or Ba-LPS activation of human T cells. In addition, several gp120-subunits are being tested for their ability to generate cross neutralizing antibodies after conjugation to Ba and Ba-LPS.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01009-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Detection of Viruses in Cell Substrates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: C.J. Marcus-Sekura, Ph.D. Chief, LDNAVR, DV, CBER
P.F. Zhang, Ph.D., Visiting Associate, LDNAVR, DV, CBER
M. Klutch, Microbiologist, LDNAVR, DV, CBER
J. Muller, LPENDEA, DV, CBER

COOPERATING UNITS *(if any)*

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is of interest to CBER whether cell lines used for product production contain virus or virus-like particles or viral nucleic acid sequences or support the growth of viruses infectious for humans. In previous studies, we used electron microscopy to examine endogenous retroviral-like particles found in the Chinese Hamster Ovary (CHO) cell line which is used as the cell substrate for several recombinant products made in CHO cells. CHO cells contain endogenous retroviral-like particles visible by electron microscopy. We attempted to determine whether growth factors or cytokines (which might be manufactured in CHO cells) might affect these endogenous particles. The results indicated increases in particles from 30-100% after treatment of CHO cells with several cytokines (GM-CSF, IFN, TNF) or with the phorbol ester TPA. A manuscript describing these findings has been published in In Vitro Toxicology (1991). We have recently approached another cell substrate issue. Since many new recombinant biological products are produced using the Baculovirus expression system and use an insect cell line called Sf9; and relatively little is known about other viruses which can infect Sf9 cells, particularly those with human host range, we recently initiated a study to determine whether any viruses pathogenic for humans can infect Sf9 cells. We have examined a series of viruses for their ability to infect Sf9 cells, and protocols for evaluating whether Sf9 cells are free of viral contamination and suitable for use for production of biological products have been developed. We evaluated the ability of Sf9 cells to be infected by fifteen viruses chosen to represent several classes of arboviruses as well as several additional viruses with wide host range. St Louis Encephalitis virus (SLE) a flavivirus, can productively infect the SF9 cells. Results with three other flavivirus, attenuated strains of Yellow Fever (17D vaccine strain) (YF), Dengue virus 1 (DEN-1 and 2 (DEN-2), suggest possible low level infection which cannot be sustained. SLE causes CPE in the Sf9 cells (10-20% giant cells). YF, DEN-1, and DEN-2 do not produce obvious CPE in the Sf9 cells. SLE can also be detected in SF9 cells by immunofluorescence and electron microscopy. Plaque assays on Vero cells using filtered supernatants from SLE-infected Sf9 cells are positive. Furthermore, many of the viruses we tested apparently can survive for long periods in cell culture without causing apparent CPE, several can survive up to 8 weeks in media alone. This may present risks of contamination for products produced in Sf9 cells if appropriate testing is not instituted.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01010-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mechanistic Approaches to HCMV mtrII and mtrIII-induced transformation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Razzaque, PhD, Senior Staff Fellow, LDNAVR, DV, CBER
O. Williams, Microbiologist, LDNAVR, DV, CBER
J. Wang, MD, Visiting Fellow, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

Dr. Clinton Jones, Associate Professor, Department of Veterinary Science, University of Nebraska, Lincoln, Nebraska 68583

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.9	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human cytomegalovirus has been linked to numerous types of human malignant diseases such as neuroblastoma, prostate cancer, cervical cancer and colon carcinoma. The virus is reactivated in AIDS and in transplant patients. Its transforming potential is of great concern in vaccine development. We reported two transforming domains in the long unique segment of human cytomegalovirus (HCMV) genome: i) mtrII (980 bp) and ii) mtrIII (7.5 kb). Transforming functions were studied by generating deletion clones, transfecting the clones by DNA mediated gene transfer into mouse 3T3 or Rat-2 cell lines and assaying the cells for anchorage independent growth in agarose and for tumorigenicity in mice. The mtrII region was found to contain 3 open reading frames (ORFs) (79, 83 and 34 aa) by DNA sequence analysis. We also reported the promoter activity in the upstream region of ORFs in mtrII using CAT assays and the detection of mtrII transcripts in the transformants. Similar mRNA species were also detected in HCMV infected cells. Along these lines, mtrII transforming activity was also tested in human cells. We recently identified that mtrII was capable of transforming human epidermal keratinocyte cell line (RHEK-1) to tumorigenicity, and the tumors were diagnosed as poorly differentiated carcinoma. Further experiments will be necessary to identify the role of the ORFs in transformation by generation of further deletion clones or mutagenesis of the ORFs. Also mapping of the transcripts will be attempted to identify whether the same gene is expressed in both the transformed and the infected cells. Regarding mtrIII (7.5 kb) transforming domain, the CMV major immediate early gene (IE-1) is contained within it. This IE-1 gene is a candidate subunit vaccine. Our deletion analysis localized the transforming activity of mtrIII to a 2.1 kb region beyond IE-1 gene. This region also contained several ORFs which are currently under investigation for their role in transformation.

These studies will be applicable to generate a safe live attenuated CMV vaccine, a subunit vaccine and most importantly to use CMV as a cloning vehicle for gene therapy.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01011-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Oncogenic potential of human herpesvirus-6 (HHV-6)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Razzaque, PhD, Senior Staff Fellow, LDNAVR, DV, CBER
O. Williams, Microbiologist, LDNAVR, DV, CBER
J. Wang, MD, Visiting Fellow, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

Johng S. Rhim, PhD, Laboratory of Cellular and Molecular Biology, National Cancer Institute, Bethesda, MD 20892. Dr. Janus Luka, Associate Professor, Department of Pathology and Microbiology, University of Nebraska, Omaha, NE 68198

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.3	0.8	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human herpesvirus-6 (HHV-6) has been proposed to be associated with B-cell lymphoma and other lymphoproliferative disorders especially in immunosuppressed settings. The virus (GS strain) was first isolated in 1986 from patients with AIDS and other lymphoproliferative disorders. To elucidate its role in human malignancies, we examined its transforming potential. Using our established assay system for gene transfer and transformation, we first reported that HHV-6 genome (170 kb, GS isolate) and two subgenomic clones pZVH14 (8.7 kb insert) and pZVB70 (21 kb insert) could transform NIH 3T3 cells to tumorigenicity. Two other HHV-6 isolates, Z-29 (CDC isolate from AIDS patient) and GD (isolated from chronic fatigue syndrome) were tested for transforming activity to understand whether it is a common property with HHV-6. Like GS isolate, genomic DNAs of GD and Z-29 isolates also transformed 3T3 cells to tumorigenicity. Restriction digestion of the viral DNAs indicated distinct difference between Z-29 and GS or GD isolates and a minor difference between GS and GD. Z-29 DNA seemed to be more tumorigenic than GS or GD DNA. We are currently performing the molecular biological analysis of the tumors to identify the gene sequences involved in transformation and whether a common transforming gene exists in these isolates.

We also investigated whether HHV-6 can transform human cells to establish its relationship to human malignancies. We found that the same two subgenomic clones pZVH14 and pZVB70 that could transform NIH 3T3 cells, were also capable of tumorigenic conversion of human epidermal keratinocyte cell line (RHEK-1). Southern blot analysis indicated the presence of pZVH14 DNAs in the transformed and tumor cells. Chromosome analysis identified the tumors to be of human origin. Tumor cells showed some chromosomal aberrations. Tumors were diagnosed as poorly differentiated carcinomas by histological analysis. Further studies are in progress to map the transforming genes and to identify the mechanism of transformation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01012-03 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

HHV-6 DNA Induced Tumors and Tumor Infiltration Lymphocytes (TIL)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: A. Razzaque, PhD, Senior Staff Fellow, LDNAVR, DV, CBER
O. Willilams, Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

Raj K. Puri, MD, PhD, Division of Cytokine Biology, FDA, CBER

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DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.1	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We reported that HHV-6 DNA transformed NIH 3T3 cells produced tumors in nude mice and these tumor cells were tumorigenic in immunocompetent NIH Swiss mice. Tumor specific TIL could be generated from HHV-6 genomic DNA induced tumors. We recently reported that a HHV-6 subgenomic clone pZVH14-induced tumor cells (14-2T) metastasized to the lungs upon intravenous injection into immunocompetent mice. TILs could also be generated from these 14-2T derived tumors, however, these TILs lacked tumor specific lytic property. This project involving generation of TILs will be discontinued temporarily because of the lack of personnel and appropriate resources. However, identification and mapping of the transforming gene will be pursued in other projects.

Publications related to project Z01 BF 01012-03 LDVR

Razzaque A, Puri RK. Human herpesvirus-6: tumorigenicity and tumor infiltrating lymphocytes, Cancer Lett 1992; 61:111-118

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01013-02 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of HIV-1 env-mediated membrane fusion and syncytia formation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. Golding Ph.D., Supervisory Microbiologist, LDNAVR, DV, CBER
J. Manischewitz, Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

D. Dimitrov Ph.D. Visiting Professor, LMMB, NCI, NIH
R. Blumenthal Ph.D. Senior Investigator, LMMB, NCI, NIH

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

Immunology

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a sensitive assay to examine the early stages of HIV-1 env mediated cell membrane fusion which is based on redistribution of fluorescent dyes between HIV-1 env-expressing cells and CD4-positive adjacent cells, monitored by fluorescence video microscopy.
This assay demonstrated that the terms cell fusion and syncytia formation can not be interchangeably used. Cell fusion and syncytia formation followed different kinetics. Cell fusion started at 15 min. and reached plateau at 90 min., while syncytia formation first appeared at 2 hr, and reached a plateau at 5-6 hrs. Furthermore, fusion occurred under conditions (i.e. cell ratios) which did not favor syncytia formation (similar to the in vivo situation).
This assay was used to re-examine the role played by the adhesion molecules LFA-1/ICAM-1 during HIV-1 env-mediated cell fusion. LFA-1 -deficient B cell lines (from patients with LAD), and T cells (generated in our laboratory by chemical mutagenesis) were used in the study.It was found that the LFA-1 adhesion molecules do not play a role during the early events of HIV-1 env-mediated cell fusion, but do contribute to later events leading to syncytia formation.

In a separate project, we studied the fusion between human B cell lines end HIV-1 env-expressing cells. We identified a fusion mechanism which is initiated by CD4-gp120 interactions, but greatly enhanced by the Ig receptors on B cells, providing they bind to the CD4-binding-region on the gp120 envelope. This study provides a mechanism for the selective fusion and elimination of B-cells expressing anti-gp120 Ab receptors.

This assay will be used to screen drugs which may block early stages in HIV-1 cell membrane fusion.

Publications related to project Z01 BF 01013-03:

Dimitrov DS, Golding H, Blumenthal R. Initial Stages of HIV-1 env-mediated cell fusion monitored by a new assay based on redistribution of fluorescent dyes, AIDS Res Hum Retroviruses 1991; 7:799-805.

Golding H, Dimitrov DS, Blumenthal R. LFA-1 adhesion molecule is involved in HIV-1 env-mediated syncytia formation but not in the early stages of cell membrane fusion, AIDS Res Hum Retroviruses 1992; (in press).

Golding H, Dimitrov DS, Blackburn R, Manischewitz J, Blumenthal R, Golding B. Fusion of human B cell lines with HIV-1 env-expressing T cells can be enhanced by antigen-specific immunoglobulin receptors: implication for elimination of gp120-specific B cells in vivo, J Immunol 1992; (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01014-02 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CMV induction of TNF-alpha in infected tissues

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.F. Manischewitz, Microbiologist, LDNAVR, DV, CBER
H. Golding, Ph.D. Supervisory Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

P.D. Smith M.D., Senior Investigator, LI, NIDR, NIH

LAB/BRANCH

Laboratory of DNA virus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.8	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CMV is a major cause of inflammatory organ disease in immunosuppressed patients. To elucidate the mechanisms of CMV-induced inflammation, tumor necrosis factor (TNF-alpha) was studied in AIDS patients with CMV colitis. In situ hybridization techniques were used on colonic mucosa from AIDS patients and normal controls. Positive hybridization of TNF-alpha probe to mRNA was obtained with colonic mucosa from patients, and was associated with CMV, but not HIV-1 infected cells. Also, a patient derived isolate of CMV induced primary human blood monocytes to express TNF-alpha mRNA and increased the levels of secreted TNF-alpha. Thus, CMV induction of TNF-alpha may play a central role in CMV-induced inflammation. Furthermore, because of the known effects of TNF-alpha on the expression of HIV-1 provirus, CMV may be a co-factor in activation of latent HIV-1, without the requirement for co-infection of cells by the two viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01015-02 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

EBV in HIV vaccine protocol.Epidemiological studies of HTLV I&II in endemic areas

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G.R.Armstrong, Biologist, LDNAVR, DV, CBER
C.J. Marcus-Sekura, PhD, Acting Deputy Director, DV, CBER
M. Ruta, PhD, D.T.S., L.R., CBER
P.H. Levine, MD, DCE, EEB, NCI
A. Pajeau, MD, NEB, NINDS
R.A. Sausville, D.T.S., L.R., CBER

COOPERATING UNITS *(if any)*

Neuroepidemiology Branch, National Institute of Neurological Diseases and Stroke
Viral Epidemiology Section, Div of Cancer Etiology, National Cancer Institute

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Because of the manifestations of the Epstein-Barr Virus (EBV) in the normal and immunosuppressed host (fulminant lymphoproliferative syndrome versus malignant lymphoma-like lesions versus typical, acute infectious mononucleosis) and the known association of EBV with African Burkitt's lymphoma and nasopharngeal carcinoma, we have been conducting a diversified investigation into the role of EBV high-risk patients who are enrolled in the AIDS vaccine study.

Research studies have disclosed a relatively high prevalence of HTLV-I/II seropositivity in American Indians, notably in the Guaymi tribes in Central America, Venezuelan Indians, Alaskan Indians, among North American plains Indians (Pueblos and Zunis) and in the Seminoles and Mikosukees in Florida. In addition, HTLV-II antibody has been found to be at a higher than normal incidence in Indians tested at blood banks in the Albuquerque area. Recent studies have suggested an over-representation throughout central New Mexico of Hispanics and American Indians among seropositive blood donors including those shown to be infected with HTLV-II, who denied travel to endemic areas, intravenous drug abuse or any known risk factor for HTLV infection.

An epidemiologic study is planned of HTLV-I/II seroprevalence and neurologic symptoms in Seminole Indians and related tribes in the Southeastern United States, Oklahoma, other North American tribes identified as possible sources of endemic retrovirus and certain islands in the Caribbean. Our efforts would be expedited if we were able to identify the tribes of American Indians which show the highest prevalence of HTLV-I/II seropositivity and if this may be attributed to migration or to familial transmission. The accomplishment of our goals as well as the orientation and direction of our study would be facilitated if we were able to perform serological testing of volunteers from Native American employees as well as non-Indians in the Parklawn Building Indian Health Service. With antibody results, we would know which tribes may show increased prevalence.

The required assays include an initial ELISA screen for HTLV-I/II which, if positive would be followed by HTLV-I Western Blot. In the event of a negative or borderline HTLV-I Western Blot, PCR would be performed for HTLV-II.

Publications related to project Z01 BF 01015-02 LDVR

Wittek AE, Mitchell CD, Armstrong G, Albini A, Martin GR, Seemann R, Levenbook IS, Wierenga DE, Ridge J, Dunlap RC, Lundquist M, Steis RG, Longo DL, Muller J, Quinnan Jr GV. Propagation and properties of Kaposi's sarcoma-derived cell lines obtained from patients with AIDS: similarity of cultured cells to smooth muscle cells, AIDS 1991; 8:303-310.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01016-01 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Kinetics of sCD4 interactions with HIV-1 env-expressing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: H. Golding Ph.D., Supervisory Microbiologist , LDNAVR, DV, CBER
J. Manischewitz, Microbiologist, LDNAVR, DV, CBER
R. Blackburn, Biologist, LDNAVR, DV, CBER, FDA

COOPERATING UNITS *(if any)*

D. Dimitrov, Ph.D., Visiting Professor, LMMB, NCI, NIH
R. Blumenthal, Ph.D, Senior Investigator, LMMB, NCI, NIH

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

Immunology

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The high affinity interaction between the HIV-1-envelope (gp120/41) and its receptor CD4, was the basis for several clinical protocols in which sCD4 or chimeric CD4-Ig(Fc) were used in treatment of HIV-1 seropositive patients. These trials did not result in success thus far. Our goals were : (a) To study the kinetics of the interactions between sCD4 and HIV-1 env-expressing cells, in relation to subsequent events leading to cell fusion and inhibition of syncytia formation.
Methods: The association and dissociation rates of sCD4 interactions with env-expressing cells, and the quantification of sCD4-induced gp120 shedding was determined by a quantitative flow cytometry assay. Syncytia inhibition was measured in the continuous presence of sCD4, or after washing of cells following pre-incubation with sCD4.
Results: The kinetics of syncytia formation inhibition correlated with sCD4 binding when sCD4 was maintained during the culture. However, when the env-expressing cells were preincubated with sCD4 and then washed to remove unbound sCD4, no syncytia inhibition was observed, even following sCD4-induced shedding of >50% of surface gp120 molecules.
Conclusions: The lack of syncytia inhibition seen after removal of unbound sCD4, even after pre-incubation of cells under saturation and gp120-shedding conditions, were explained by the rapid dissociation of the bound sCD4 from the env-expressing cells (k/min: 1.9×10^{-2}), allowing cell fusion to continue. It also suggested that sufficient numbers of fusogenic molecules remained on the sCD4-treated cells. These studies are important for understanding HIV-1env-mediated cell fusion and AIDS therapy with CD4 derivatives, or other agents (e.g. anti-HIV-1env monoclonal antibodies).

PHS 6040 (Rev. 5/92)

Publications related to project Z01 BF 01016-01 LDVR:

Dimitrov DS, Hillman K, Manischewitz J, Blumenthal R, Golding H. Kinetics of soluble CD4 binding to cells expressing human HIV-1 envelope glycoprotein, J Virol 1992; 66:132-138.

Dimitrov DS, Hillman K, Manischewitz J, Blumenthal R, Golding H. Correlation between kinetics of soluble CD4 interactions with HIV-1-env-expressing cells and inhibition of syncytia formation : Implications for mechanisms of cell fusion and therapy for AIDS. AIDS 1992; 6:249-256.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01017-01 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cooperating activity of HPV-16 and HSV-2 or HCMV DNA in transforming human cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Abdur Razzaque, PhD, DV, CBER

COOPERATING UNITS *(if any)*

Dr. Clinton Jones, Department of Veterinary Science, University of Nebraska, Lincoln, Nebraska 68583

LAB/BRANCH

Laboratory of DNA Virus Research

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INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.2	PROFESSIONAL:	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Human papilloma virus type 16 (HPV-16), herpes simplex virus type 2 (HSV-2) and human cytomegalovirus have been linked to cervical cancer. We examined whether HSV-2 and HPV-16 DNA sequences can transform early passage human cells. Gingival fibroblasts derived from a normal individual (N-16) and from a hyperplastic gingival tissue of an epileptic patient (R-30) treated with phenytoin were transfected with HPV-16 DNA alone or in combination with HSV-2 DNA. HPV-16 DNA efficiently immortalized R-30 cells but not N-16 cells. R-30 cells co-transfected with HPV-16 and HSV-2 DNAs were more aneuploid and grew better in soft agar than R-30 transfected with HPV-16 DNA alone. Both HSV-2 and HPV-16 DNA sequences were detected by Southern blot and PCR analysis in transformed cells. We have published a paper with these data. We will examine the expression of E6 and E7 genes of HPV and activation of cellular oncogenes in these transformants. We would also like to pursue similar studies with HCMV DNA sequences. These data suggest that HPV-16 and HSV-2 DNA sequences can cooperate in multistep process of transformation.

Publication related to project Z01 BF 01017-01 LDVR

Dhanwada KR, Veerisetty V, Zhur F, Razzaque A, Thompson KD, Jones C. Characterization of primary human fibroblasts transformed by human papilloma virus type 16 and herpes simplex virus type 2 DNA sequences, J Gen Virol 1992; 73:791-799.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01018-01 LDVR

PERIOD COVERED

October 1, 1991 through September 30 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

CMV-infected astrocytes produce TGF-beta

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.F. Manischewitz, Microbiologist, LDNAVR, DV, CBER
H. Golding, Ph.D., Supervisory Microbiologist, LDNAVR, DV, CBER

COOPERATING UNITS *(if any)*

P.D. Smith, M.D., Senior Investigator, LI, NIDR, NIH

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

Immunology

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD, 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

CMV infection of the central nervous system in immunocompromised adults, such as AIDS patients, can cause devastating neuropathology. Since cytokines play a pivotal role in inflammatory responses and may be important mediators of central nervous system dysfunction, we investigated the role of transforming growth factor (TGF-Beta) in the pathogenesis of CMV encephalopathy. Brain tissue from AIDS patients with CMV cerebritis, but not normal subjects, contained TGF-beta detected by immunoperoxidase staining. TGF-beta staining could be localized within or near astrocytes, some of which were infected with CMV. To determine whether CMV infection of astrocytes could induce production of TGF-beta, we incubated murine astrocytes with murine CMV in vitro. The infected astrocytes were assayed for TGF-beta mRNA, and the astrocyte culture supernatants were assayed for CMV and TGF-beta. It was found that the astrocytes were productively infected with the murine CMV, expressed TGF-beta mRNA, and secreted TGF-beta peptide.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 01019-01 LDVR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular Biology of Herpes Simplex Virus Latency

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P.R. Krause Senior Research Investigator LDVR/DV/CBER/FDA

COOPERATING UNITS *(if any)*

Medical Virology Section, LCI/NIAID/NIH (S.E. Straus); Division of Infectious Diseases, Children's Hospital Research Foundation, Cincinnati, OH (L. Stanberry)

LAB/BRANCH

Laboratory of DNA Virus Research

SECTION

INSTITUTE AND LOCATION

Division of Virology, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The function and regulation of the herpes simplex virus type 2 (HSV-2) latency-associated transcript (LAT) was studied *in vivo* and *in vitro*.

A. LAT regulation. Promoter sequences directing the transcription of the HSV-2 LAT were identified in transient expression assays and functionally compared in tissue culture with those for the HSV-1 LAT. The HSV-2 LAT promoter is stronger than the corresponding sequences from HSV-1 in all cell lines tested, but the relative activity of the two promoters varies among cell lines. Both HSV-1 and HSV-2 LAT promoters respond to inducers of cAMP and to HSV immediate-early gene ICP0.

B. LAT function. A mutant of HSV-2 lacking the promoter sequences for the LAT (and thus unable to produce the LAT) and a rescued virus in which these sequences were restored were constructed. Characteristics of acute and latent infection in tissue culture and in guinea pigs were compared among the wild-type, mutant, and rescued viruses. During acute infections, the three viruses exhibited no appreciable difference in kinetics of replication or severity of lesions. Deletion of the LAT promoter sequences led to a marked decrease in spontaneous recurrences in guinea pigs latently infected with the mutant virus as compared with those infected with wild-type or rescued virus. Thus, the LAT gene plays a role in spontaneous reactivation from HSV-2 latency.

The significance of this project lies in the identification of viral mechanisms important in establishment and reactivation from latency. An understanding of these mechanisms is critical for a complete evaluation of biological products which may influence the latent state.

Publications related to project Z01 BF 01019-01 LDVR:

Krause PR, Ostrove JM, Straus SE. The nucleotide sequence, 5' end, promoter domain, and kinetics of expression of the gene encoding the herpes simplex virus type 2 latency-associated transcript, J Virol 1991; 65:5619-23.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 02002-06 LRVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cellular and humoral immune responses to rhinovirus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. A. Levandowski, M.D., Senior Staff Fellow, LRVD, DV, CBER
V. Tyson-Medlock, LRVD, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Respiratory Virus Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.2	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antisera from guinea pigs immunized against individual human rhinovirus (HRV) serotypes were used to determine reactivity in an immunoblot system with the capsid surface peptides (VP1, VP2, VP3) of HRV serotype 14 (HRV14) in a continuation of studies begun with HRV2. HRV14 and HRV2 were purified by CsCl gradient, separated into capsid peptides by electrophoresis through polyacrylamide and transferred to nitrocellulose membranes for immunoblotting. Serum IgG binding was identified by immunoperoxidase methodology. Only the antiserum specific for HRV14 neutralized HRV14 and only the antiserum for HRV2 neutralized HRV2. However, the majority of the antisera against 53 individual serotypes reacted with HRV2 and HRV14. Nine of the antisera reacted with neither HRV and five of the antisera reacted with both. Thirty of the 53 antisera reacted only with HRV2 and 19 reacted only with HRV14. The pattern of reactivity was best correlated with a defined spectrum of susceptibility of the HRV serotypes to antiviral chemicals which stabilize the rhinovirus capsid by binding to a relatively conserved amino acid sequence occupying a fold in the capsid surface of VP1. For both HRV 14 and HRV2, the antibodies identified were directed predominantly against antigenic determinants of VP1, and only infrequently against VP2 or VP3. The data suggest that immunogenic antigenic determinants have been conserved in the evolution of HRV. The identification of antisera for serotypes which react with capsid peptides of both HRV14 and HRV2 or with neither HRV suggests that the antigenic determinants identified may coexist in HRV representing transitions between HRV14 and HRV2, and that unrelated antigenic representing further evolution may be present.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 02003-04 LVRD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influenza Vaccine Development and Use

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. A. Levandowski, M.D., Senior Staff Fellow, LRVD, DV, CBER
M.S. Williams, Biologist, LRVD, DV, CBER
E. Staton, Biology Lab Tech (Micro), LRVD, DV, CBER
W. B. Barthlow, Jr., Biology Lab Tech (Micro), LRVD, DV, CBER
D. P. Offringa, Biologist, LRVD, DV, CBER

COOPERATING UNITS *(if any)*

University of Colorado Medical Center, Denver

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Laboratory of Respiratory Virus Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
 ☒ (a1) Minors
 ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Young children and infants often respond poorly to immunization with inactivated influenza virus vaccines. Previous studies have suggested that the means and quality of the primary exposure to influenza virus antigens affect the level of antibodies and the probability of protection produced by inactivated influenza virus vaccines. In order to study the effects of route of immunologic priming, serum was collected before and three weeks after administration with a B/Panama/45/90 vaccine from 36 children and infants with defined histories of influenza virus infection and immunization. Thirteen of the children (age 18 ± 8 months) had neither been previously vaccinated or infected; 16 (age 24 ± 8 months) had been immunized in the previous year with B/Yamagata/16/88 (a strain closely related to B/Panama/45/90; 7 (age 20 ± 10 months) had been infected with P/Panama/45/90 as shown by virus isolation (5 children) or seroconversion (2 children). Before immunization, none of the uninfected/unimmunized children had a detectable titer of a hemagglutination inhibition (HI) antibodies (GMT <1:16), but only 50 % of the previously immunized children (GMT <1:16) and all of the previously infected children (GMT = 1:48) had detectable HI antibodies. After immunization the GMT for HI antibodies rose to 1:64 in the unimmunized/uninfected group, to 1:91 for the previously immunized group, and to 1:312 for the previously infected group ($p<0.01$, one way ANOVA). In addition, there was a significant difference ($p<0.05$, t-test) in the post immunization GMT for HI antibodies in comparison of the previously immunized children with (post GMT = 1:140) and without (post GMT = 1:54) detectable antibodies before reimmunization. These results indicate that natural infection with an influenza B virus is a more potent means of immunologic priming with longer duration of antibody titers and a greater anamnestic response to subsequent antigenic challenge as compared to administration of inactivated vaccine. The results reinforce the suggestion that a separate strategy of immunization should be developed for young children and infants.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 02004-04 LRVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigenic and Molecular Characterization of the Fusion Glycoprotein of RSV

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.A. Beeler, MD, Senior Staff Fellow, LRVD, DV, CBER
R.L. Booth, Biologist, LRVD,DV,CBER
M.Perkins, MD, Fort Worth Children's Hospital, Ft. Worth Texas
B.R. Murphy, MD, Head, Respiratory Virus Section, LID, NIAID, NIH
A. Komoriya, PhD, Division of Cytokine Biology, CBER

COOPERATING UNITS *(if any)*

Respiratory Virus Section, LID,NIAID,NIH

LAB/BRANCH

Laboratory of Respiratory Virus Diseases

SECTION

INSTITUTE AND LOCATION

DV,OBR,CBER,FDA,Bethesda, Maryland

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	0.96	0.24

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Respiratory syncytial virus is the most common cause of lower respiratory tract disease in young children worldwide. The F glycoprotein of this virus is one of the important targets of a protective immune response; however, immunity is incomplete and repeated infections occur. Previously, a detailed map of the neutralization epitopes was developed based on the ability of a panel of murine anti-F monoclonal antibodies to neutralize 14 monoclonal antibody resistant mutant viruses of the A2 strain. The reactivity of the F monoclonal antibodies with the panel of F MARMs in ELISA was also assessed in order to corroborate the operational map previously developed and to demonstrate a direct correlation between resistance to neutralization and absence of antibody binding. In most cases MARMs did not bind the selecting antibody and verified the idea that escape from neutralization was due to specific mutations in the contact residues within the F epitope. In addition, 25 neutralizing and non-neutralizing F MAbs have been biotinylated and used in competitive binding studies to identify at least 6 antigenic sites on the F glycoprotein. These biotinylated MAbs were also used to develop an antigenic site blocking assay in order to analyze the immune response to specific regions on RSV-F following infection or immunization. Preliminary studies have shown that post-infection human and cotton rat sera that have similar neutralization titers, vary in the repetoire of their anti-F antibody response. During the coming year additional studies will be done to evaluate individual immune responses to the F glycoprotein following natural infection or immunization. As an extension of this work, the reactivity of F MAbs with synthetic peptides will be evaluated in order to develop an epitope specific site-directed ELISA assay.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 02005-02 LRVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sequencing of PCR Amplified cDNA of the GENE for the RSV Fusion Glycoprotein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.A.Beeler, MD, Senior Staff Fellow, LRVD, DV, CBER
R.L.Booth, Biologist, LRVD, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Respiratory Virus Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.75	0.2	0.55

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Antigenic variation of respiratory syncytial virus may play an important role in the development of disease in newborn seropositive infants and allow reinfection of older children to occur. Random genetic and antigenic heterogeneity among circulating RSV isolates may be an important obstacle to vaccine development. In addition live attenuated viruses used as vaccines are susceptible to the same type of instability and production of these vaccines could result in virus strains with altered immunogenic properties when they are compared to the parent or seed virus. Previously, this laboratory demonstrated the ability to amplify RSV-F gene sequences by the polymerase chain reaction. Direct sequencing of the PCR amplified cDNA proved to be a rapid, reliable and accurate method for determining the F gene sequences and mutations in the F glycoprotein of 14 monoclonal antibody resistant mutant viruses were identified. The biological activity of the MARMs *in vivo* was also determined as a part of this project. Replication of the MARMs in the upper and lower respiratory tract of cotton rats was compared to the replication of the the parental A2 strain. Several of the F MARMs were attenuated in the lower respiratory tract suggesting that specific mutations in the F glycoprotein may contribute to the attenuation phenotype of these viruses. Furthermore, these viruses can be considered as candidates for a live attenuated viral vaccine. In addition some of the attenuated MARMs had altered reactivity with G specific monoclonal antibodies in ELISA suggesting that mutations in this glycoprotein might also contribute to the attenuation phenotype. In order to identify mutations in the G glycoprotein of these viruses, the sequence for the G gene of the parental A2 strain has been determined using G specific primers and the methods described above. During the coming year we hope to more completely characterize the attenuated MARMs. First, the sequence for the G glycoprotein will be determined. Second, in collaboration with Dr.Brian Murphy, LID, NIAID, replication of the MARMs in primates will be evaluated.

PHS 6040 (Rev. 5/92)

PATENT related to project Z01 BF 02005-02 LRVD

Beeler JB, Murphy BR, Coelingh KC, Perkins M, Chanock RM. US Patent pending: Live attenuated strain of respiratory syncytial virus for use as vaccine: monoclonal antibody resistant mutants, April 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 02006-02 LRVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immune response to Formalin Treated Respiratory Syncytial Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: J.A. Beeler, MD, Senior Staff Fellow, LRVD, DV, CBER
R.L. Booth, Biologist, LRVD, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Respiratory Virus Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.3	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Previous attempts to develop a vaccine that would prevent disease associated with RSV infection have failed. In particular, a formalin treated, whole virus vaccine, known as the Lot 100 Pfizer vaccine, not only failed to protect but also produced enhanced disease when immunized children subsequently underwent natural infection with RSV. The exact cause of the lack of protective immunity and the potentiated disease are not completely understood. Serological markers that could predict the ability of a vaccine to protect against infection or identify the potential for enhanced disease would provide a safety test for the regulation of RSV vaccines. Investigators in other laboratories have suggested that there was an imbalance in the immune response following immunization that resulted in high titers of RSV specific binding antibody but little antibody that could neutralize virus or inhibit fusion of infected cells. Previous work in this laboratory demonstrated in an ELISA assay that formalin treated virus lost reactivity with F specific neutralizing MAbs but maintained or had enhanced reactivity with non-neutralizing F and G MAbs. In order to extend these findings, we have biotinylated 25 neutralizing and non-neutralizing F MAbs and 8 G MAbs that identify a minimum of 6 and 2 antigenic sites on the F and G glycoproteins, respectively. These biotinylated monoclonal antibodies were also used to develop an antigenic site blocking assay that can detect variations in the immune response to specific antigenic sites on the viral glycoproteins. During the coming year we will compare the antibody response of cotton rats infected intranasally with RSV to the response of animals immunized with a formalin inactivated RSV vaccine known to be associated with enhanced disease. The antigenic site blocking assay will also be used to evaluate the immune response of the children who received the Lot 100 vaccine. These studies will be done in collaboration with Dr. Mark Connors and Dr. Brian Murphy, LID, NIAID, who have the archived collection of animal and human sera.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 02007-02 LVRD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Influenza reassortant virus: biology and genetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R. A. Levandowski, M.D., Senior Staff Fellow, LRVD, DV, CBER
D. P. Offringa, Biologist, LRVD, DV, CBER
M. S. Williams, Biologist, LRVD, DV, CBER
W. B. Barthlow, Jr., Biology Lab Tech (Micro), LRVD, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Respiratory Virus Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	0.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Influenza virus vaccine candidate strains with greater yield potential than the naturally occurring wild type strains of influenza A virus have been produced by reassorting the wild type strain with a strain of influenza A/Puerto Rico/8/34 (PR8) with the ability to confer the "high yield" phenotype. The hemagglutinin (HA) and neuraminidase (NA) of a wild type strain are selected by suppressing the progeny virions bearing the HA and NA of PR8 by incubation in the presence of an antiserum produced against PR8. With this strategy, the possibility exists that a minor component of the resulting virions may still include the genotype or phenotype of PR8 HA or NA. In order to develop a sensitive method for identifying whether the PR8 genome may be persistent, the polymerase chain reaction (PCR) technique has been adapted for identification of HA and NA genes from H1N1 and H3N2 viruses. The specificity of the primers chosen for DNA polymerization was first confirmed for both HA's and NA's using a battery of H1N1 viruses and H3N2 viruses. Subsequently, H1N1 and H3N2 viruses were mixed in varying ratio in order to determine the limit of detection of the method for NA. With a single session of 30 cycles of polymerization, it was possible to detect 1 N2 virion in 1000 N1 virions. With an additional session of 30 cycles using either the same set of primers or a set of primers for a sequence contained within the product from the first set of primers, it was possible to increase the sensitivity by 10 fold, although not consistently. Thus, by PCR it was possible to specifically identify a minor component at ratios of 1:1000-1:10000. In marked contrast, the standard method of identification of neuraminidase by inhibition of enzymatic activity with a "specific" antibody, was not consistently capable of identifying mixtures of N1 and N2 virions even at ratios of 1 part in 10. The results indicate that PCR is a more sensitive method of detection of genetically mixed influenza virus populations, and that PCR techniques can be very useful in a program to produce reassortant viruses.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03005-03 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Electron Microscopy Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Muller, PhD, Cell Biologist, LPD, DV, CBER
M. Lundquist, MS, Chemist, LPD, DV, CBER
J. Kort, MD, PhD, Immunologist, U of MD Cancer Center, Dept of Pathology
T. Khavkin, PhD, Interferon Sciences, Inc, New Brunswick, NJ

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 2.0	PROFESSIONAL: 1.7	OTHER: 0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues X
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

(1) MAIDS is a lymphoproliferative, immunosuppressive disease of C57BL/6 mice caused by a replication-defective c-type retrovirus (LP-BM5). Adherent cells isolated from the peritoneal cavity and seminal fluid of infected mice were examined by electron microscopy and FACS. Greater than 90% of adherent cells were of macrophage morphology and phenotype. They were found to be expressing virus as early as 1 week after virus inoculation with the number of infected cells rising as disease progressed. As few as 10^5 adherent cells from infected mice were capable of inducing disease in 100% of naive mice when injected I.P. These studies show that cells of the macrophage/monocyte lineage are an important reservoir for LP-BM5 and may be involved in the sexual transmission of MAIDS.

(2) U937 cells were exposed to either standard Sendai virus stock (St) or to one containing St and defective interfering particles (StDi). Electron microscopic analysis showed that infection with St leads to the abundant synthesis of nucleocapsid material in the cytoplasm of most cells. Production of extracellular, formed viral particles, however, is small. Despite the substantial burden of viral products, cells appear relatively healthy and the eventual death of heavily infected cells occurs by necrosis. In contrast, infection with StDi results in very little nucleocapsid synthesis in most cells. Nonetheless these cultures exhibit a conspicuous early CPE consisting of the widespread disintegration of many cells by the mechanism of apoptosis (programmed cell death). Apoptosis differs fundamentally from necrosis as a mechanism of cell death. DI particles have not, until now, been shown to be capable of triggering this mechanism.

(3) In cooperation with the Division of Transfusion Science, liver biopsies of experimentally infected chimps are prepared for EM and examined for evidence of infection by hepatitis C virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03006-03 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

International Collaborative Study of Polio Reagents (Antibody Reagents)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.A. Sawyer, DVM, MPH, Research Associate, LPD, DV, CBER

Others:
- P. Albrecht, MD, Chief, LPD, Director, DV, CBER
- J. McInnis, Microbiologist, LPD, DV, CBER
- A. Patel, Microbiologist, LPD, DV, CBER

COOPERATING UNITS *(if any)*

Biological Standards and Control, England; Institut Pasteur, France; Rijksinstituut voor de Volksgezondheid, Netherlands

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A standard reproducible method for determining the potency of inactivated poliovirus vaccines (IPV) in vitro would be an important advance toward the eradication of polio. Protection against polio is provided by neutralizing antibodies with "D" antigen specificity. We have developed an ELISA test in which monoclonal antibody (which is "D" specific) is used for detection. Using our reagents as the standard we examined sera from other collaborating laboratories for the ability to measure the potency of IPV. We were able to examine polyclonal capture or detection sera prepared in four different species including: rabbits, cows, horses and goats. We were also able to compare two different vaccine preparations of IPV, one made from wild poliovirus and the other from Sabin strains. Furthermore, we were able to examine polyclonal sera prepared with either the wildtype or Sabin strains of poliovirus. We have found that reagents prepared against wildtype poliovirus strains react well in the ELISA with Sabin derived IPV and vice versa. The majority of monoclonal antibodies react well with wild or Sabin derived vaccines irrespective of the strain used in their preparation. These findings suggest that the attenuated strains (Sabin) when prepared as an inactivated vaccine retain antigens in common with the inactivated wildtype strains. Several monoclonal antibodies were found to be strain specific and a few non-reactive with either strain of poliovirus in the ELISA. Whether equivalent D Ag units in Sabin strain and wild type IPV will provide the same *in vivo* immunogenicity is not yet known. An ELISA test which incorporates cross-reactive as well as strain specific monoclonal antibodies will be a useful tool for comparing the potency of wild-type and Sabin derived vaccines *in vitro*.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BF 03008-03 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Effect of Preservatives on the Stability of Inactivated Poliovirus Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.A. Sawyer, DVM, MPH, Research Associate, LPD, DV, CBER

Others:
R. Lundquist, PhD, Research Chemist, LPD, DV, CBER
P. Albrecht, MD, Chief LPD, Director, DV, CBER
J. McInnis, Microbiologist, LPD, DV, CBER
A. Patel, Microbiologist, LPD, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	0.75	0.75

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have developed a sensitive ELISA assay for measuring the potency of trivalent poliovirus vaccines using mouse monoclonal antibodies for antigen detection. During routine tests of IPV we found a vaccine lot the type 2 component of which tested very low with our standard reagent; but gave satisfactory values with another type 2 monoclonal antibody or rabbit polyclonal sera. We are collaborating with another laboratory as a separate research project to identify the site specificity of our monoclonal antibodies. We are in the process of trying to elucidate the cause for this apparent epitope specific change. We reviewed the literature for chemicals known to affect the potency of poliovirus. We examined the effect of thimerosal on the potency of IPV. Preparations were held at three temperatures 37, 25 and 4°C for 2 weeks. The preparations were then tested for potency by ELISA at 2, 10, and 14 days. Vaccine preparations held at 37 and 4°C were inoculated into mice on day 14. We found that combinations of IPV and thimerosal held for 14 days resulted in a loss of potency when kept at 25°C as well as 37°C for poliovirus types 1 and 2. Poliovirus type 3 was less sensitive to thimerosal and lost potency when the vaccine was held at 37°C only. This suggested that handling of vaccines with thimerosal is very important in terms of IPV potency. We observed that our standard type 2 monoclonal antibody appeared to be directed against an epitope that had undergone changes in the presence of thimerosal. The potency of the vaccine measured *in vitro* did not correlate with immunogenicity in mice. These findings have important implications for manufacturers of combination vaccines as they will need to consider the effect of each preservative on the antigens in the combination.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03013-02 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Reactions Following Immunization with Japanese Encephalitis Virus Vaccine

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.A. Sawyer, DVM, MPH, Research Associate, LPD, DV, CBER

Others:
P. Albrecht, MD, Chief LPD, Director, DV, CBER
P. Turkeltaub, MD, LAP, DBP, CBER
J. Kenimer, PhD, LCP, DBP, CBER

COOPERATING UNITS *(if any)*

Lab of Vector-Borne Infectious Diseases, CID, CDC, Fort Collins, Colorado (T. Tsai, MD, Chief); Dept of Epidemiology, Copenhagen, Denmark (T. Ronne, MD, Head); Navy Environmental Health Center, Dept of the U.S. Navy (S.W. Berg, MD)

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Japanese encephalitis virus is the causative agent of an acute inflammatory illness of the central nervous system found throughout eastern Asia. A formalin inactivated, mouse-brain derived Japanese encephalitis virus vaccine has been developed. A report of severe urticarial reactions associated with immunization with Japanese encephalitis virus vaccine was published in 1991 in The Lancet. The rate of severe side-effects was reported to be 0.04%. The description of the adverse reactions was similar to allergic reactions seen when booster doses of rabies vaccine were given. The Centers for Disease Control were invited to participate in an investigation of the reactors. A case-control study found that persons developing these reactions after JE vaccination were more likely to have had a past history of urticaria (after hymenoptera envenomation, drugs, physical or other provocations or of idiopathic cause). Sixteen reactors were skin tested with the vaccine causing the allergic reaction at least 5 weeks after the reaction. None of the reactors showed skin test positivity. Acute and convalescent sera were drawn for testing for IgE levels and reactivity with the vaccine. These tests are still in progress. The mechanism and cause of these reactions is still unknown. The vaccine labeling will include adequate warnings to inform physicians and recipients of the vaccine about the allergic reactions.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03016-01 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

WHO Collaborative Study of Inactivated Poliovirus Vaccines

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.A. Sawyer, DVM, MPH, Research Associate, LPD, DV, CBER

Others:
P. Albrecht, MD, Chief LPD, Director, DV, CBER
J. McInnis, Microbiologist, LPD, DV, CBER

COOPERATING UNITS *(if any)*

(List Attached) Manufacturers from: France, Belgium, Canada, The Netherlands, Sweden and New York; Control Authorities from: England, Canada, Denmark, Germany, France, Finland and Belgium

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The current tests for inactivated poliovirus vaccine (IPV) potency need standardization between laboratories. A collaborative study has been initiated to assess the suitability of candidate reference materials. Another aspect of the study is to assess the suitability of a candidate standard method for D antigen assay by ELISA, and to assess the proficiency of methods for potency estimation of IPV used by individual laboratories. The materials included in this study are two commercially prepared candidate reference materials; the primary reference PU78-02 currently used by several laboratories, vaccines from different manufacturers shown to be immunogenic in humans; IPV produced from Sabin strains and vaccines of poor in vivo potency. The study will be divided into two phases. The first phase which is in progress will involve in vitro assays and the second phase in vivo assays.

An ELISA developed at CBER using as detection antibodies monoclonal antibodies specific for D antigen is the candidate standard method. We filled and lyophilized our in-house reagents for distribution to collaborators. Several tests were performed to validate the lyophilized reagents prior to mailing. We prepared a detailed SOP which included the dilution schemes for both practice antigens and test antigens. The collaborators have received an ELISA kit containing plates, polyclonal and monoclonal antibodies and monotypic antigen of all three poliovirus types for practice together with the SOPs and the results of our tests using the monotypic antigens. The test antigens labeled A-F were mailed at a later date. Testing is in progress. We are currently evaluating the Balb/c mouse as a model of in vivo potency as a separate study. We plan to propose using Balb/c mice as a candidate standard in vivo potency method for the second phase of the collaborative study. We will be involved in the analysis and reporting of the study at each phase.

Manufacturers: Pasteur Merieux Serums et Vaccins, France (L. Peyron); Smith Kline Beecham Biologicals, Belgium (M. Duchene); Connaught Lab Ltd., Canada (J.G. Kreeftenberg); RIVM, The Netherlands (E.C. Beuvery); Lederle Laboratories, New York (J. Mezzatesta); and The National Bacteriological Laboratory, Sweden (B. Larsson)

Control Authorities: National Institute for Biological Standards and Control, England (D. Wood); Bureau of Biologics, Canada (L. Palkonyay); Statens Seruminstitut, Denmark (Ladefoged); Paul Ehrlich Institut, Germany (M. Week-Luthauann); Laboratoire National de la Sante, France (J.P. Cano); National Public Health Institute, Finland (T. Hovi); and Institute of Hygiene and Epidemiology, Belgium (E. Druyts-Voets)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03017-01 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Serologic response to Edmonston-Zagreb and Moraten measles vaccine, United States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. Albrecht, MD, Senior Supervisory Microbiologist, LPD, DV, CBER
B. Bland, Microbiologist, LPD, DV, CBER
S. Odette, Biologist, LPD, DV, CBER
L. Markowitz, MD, CDC, Atlanta, GA

COOPERATING UNITS *(if any)*

Centers for Disease Control, Atlanta, GA

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To compare the serologic response to two different measles vaccine strains and vaccination at different ages, we conducted a randomized trial of Edmonston-Zagreb (EZ) and Moraten measles vaccines in the US. Study groups included: EZ1 (4.84 TCID$_{50}$) at 6 months, EZ1 at 9 months, EZ2 (3.84 TCID$_{50}$) at 9 months, Moraten (3.79 TICD$_{50}$) at 9 months and Moraten (as combined measles, mumps, and rubella vaccine, MMR) at 12 months. Antibody (Ab) was measured before vaccination (prevacc) and 3 months postvacc by plaque reduction neutralization.

	% with Ab>1:120 postvacc, by prevacc Ab					
Vaccine group	prevacc -		prevacc +		Total	
	n	%	n	%	n	%
EZ1 - 6 mos	88	94	154	56	242	70
EZ1 - 9 mos	125	96	69	83	194	91
EZ2 - 9 mos	104	88	56	57	160	78
Moraten - 9 mos	119	95	74	72	192	86
Moraten -12 mos	120	94	16	75	136	92

Seroconversion rates for all vaccines were lower in the presence of maternal Ab. Seroconversion rates were similar in children who received high dose EZ at 9 mos and Moraten at 12 mos. While seroconversion rates were lower with Moraten at 9 mos compared with Moraten at 12 months,the differences were not statistically significant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03018-01 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Declining measles antibody titers in mothers and infants, United States.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: P. Albrecht, MD, Senior Supervisory Microbiologist, LPD, DV, CBER
B. Bland, Microbiologist
L. Markowitz, MD, CDC, Atlanta, GA
R. Demonteverde

COOPERATING UNITS *(if any)*

Center for Disease Control, Atlanta, GA
Kaiser Permanente, Los Angeles, CA

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.1	

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Younger US-born mothers are more likely to have measles vaccine-induced immunity than are older mothers and are less likely to have been boosted by exposure to wild virus. To assess whether younger mothers have lower antibody titers and whether their children lose antibody at a younger age, we determined pre-vaccination plaque reduction neutralization (PRN) measles antibody titers in infants at either 6,9, or 12 months of age and their mothers who were enrolled in a measles vaccine trial in LA, CA. Sera collected from 913 mothers (n=527), those born <1957 had a higher PRN geometric mean titer (GMT) than those born ≥1957 (4707 vs. 1592, P<0.001). Among non-US born mothers (n=386), there were no differences in GMT by year of birth (1854 vs 2142). Children born to younger US-born mothers were more likely to be seronegative than those born to older mothers at 6 months (47% vs 17%, p=0.003), 9 months (65% vs 36%, p<0.001), and 12 months (88% vs 82%, p=NS). Response rates to Moraten measles vaccine were higher in children born to younger compared with older US-born mothers at 9 months (87% vs 58%, p=0.008) and 12 months of age (94% vs 83%, p=NS). Response rates to measles vaccine in the US may be higher in infants than previously reported due to lower levels of maternal antibody in children born to younger women.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03019-01 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In vivo Potency of Inactivated Poliovirus Vaccine (IPV)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.A. Sawyer, DVM, MPH, Research Associate, LPD, DV, CBER

Others:
- P. Albrecht, MD, Chief LPD, Director, DV, CBER
- J. McInnis, Microbiologist, LPD, DV, CBER
- A. Patel, Microbiologist, LPD, DV, CBER

COOPERATING UNITS *(if any)*

None

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An international consensus as to the best method for measuring the potency of inactivated poliovirus vaccines (IPV) in vitro or in vivo has not yet been achieved. Laboratories do not agree on the most feasible animal model of potency and therefore laboratories use rats, guinea pigs, chickens or primates none of which are ideal for many reasons. In the U.S. and Canada, the monkey is required for the purpose of IPV lot release. Monkeys are given 3 injections at 7 day intervals. This hyper-immunization may obscure the antigenicity. The new purified vaccines induce an antibody response in monkeys that would rarely fail a vaccine lot even if the level of D Ag units in the vaccine was lower than would be recommended for a human dose. For these reasons we are pursuing the identification of an appropriate animal model. We have selected the Balb/c mouse for further study. Based on the manufacturer's assigned potency, serial two-fold dilutions of trivalent IPV were prepared. The potency of the first vaccine dose was equivalent to a human dose. Six groups of mice were immunized intraperitoneally with one injection. Neutralizing antibodies were measured at 21 days. The potency of the six preparations of vaccine were measured by ELISA. The correlation between the amount of antigen injected and neutralizing antibody response was R=0.90, R= 0.85 R=0.89 for poliotypes 1, 2 and 3 respectively. We are in the process of expanding this approach to confirm its usefulness.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 03020-01 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

In vitro translation of wild-type and attenuated poliovirus genomes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.E. Lundquist, Ph.D., Research Chemist, LPD, DV CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Attenuation of poliovirus neurovirulence is the result of a limited number of nucleotide changes in the poliovirus genome. A major site for attenuation (position 472) occurs in a noncoding region of the genome that is involved in the regulation of viral translation. Exactly how (and if) changes in translation result in poliovirus attenuation remain to be established.

A recent report by Mola, Paul, and Wimmer, Science 1991;254:1647-1651 suggested that an in vitro translation system derived from HeLa cells translated added poliovirus RNA in a manner that closely mimics the full viral replicative cycle in human cells. We are using this sytem to determine if wild-type and attenuated genomes exhibit different translational responses in the in vitro extract from a human cell line. HeLa cell extracts have been prepared which respond to the addition of poliovirus RNA by synthesizing poliovirus proteins in vitro. Characteristics of the response to wild type viral RNA are being evaluated and will be used to compare the response to RNA carrying the attenuated phenotype.

Elucidation of the role translation plays in the attenuation of poliovirus neurovirulence could lead to further improvements in the stabilization of the attenuation phenotype.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BF 03021-01 LPD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Stabilization of poliovirus infectivity to lyophilization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: R.E. Lundquist, Ph.D., Research Chemist, LPD, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Pediatric Diseases

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	0.8	0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Oral poliovirus vaccine (OPV) can rapidly lose potency when not maintained in a frozen state. Stabilization of OPV to less stringent storage conditions would greatly facilitate the poliovirus immunization program. Some vaccines can be lyophilized and stored in a solid state, but poliovirus infectivity is greatly diminished after lyophilization.

We have systematically evaluated different conditions and additives in search of a procedure that would allow the successful lyophilization of poliovirus. Lyophilized material was assayed by both the Elisa assay for D antigen and for infectious virus by a plaque assay. Dramative improvements in viral survival were obtained with the use of a lipid based reagent, which increased viral survival by 10^3 and resulted in 20-40% of the viral infectivity surviving lyophilization. Attempts are continuing to further increase virus stability to lyophilization and to evaluate the temporal and temperature stability of the lyophilized virus.

PHS 6040 (Rev. 5/92)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 04003-04 LRR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

B cell activation in Pathologic States

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: D.M. Klinman, MD, PhD, Senior Staff Fellow, LRR, DV, CBER
Akira Shirai, MD, Fogarty Fellow, LRR, DV, CBER
Mark Cosentino, DPM/PhD, LRR, DV, CBER
Jacqueline Conover, B.S., Technician, LRR, DV, CBER
Sylvester Daniels, Technician, LRR, DV, CBER

COOPERATING UNITS *(if any)*

Dr. David Schwartz, Johns Hopkins University
Dr. Jeffrey Kopp, NIDR

LAB/BRANCH

Laboratory of Retrovirus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5.0	3.0	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research is divided into studies concerning the regulation of i) humoral immune responses to HIV-1 envelope glycoproteins, ii) virus replication in murine models of AIDS and iii) B cell activation in retrovirus-induced and autoimmune diseases.

i) Serum and cells were obtained from mice and humans immunized with purified recombinant envelope glycoproteins from a variety of HIV-1 isolates in order to determine the number, specificity and cross-reactivity of B cells producing antibodies against gp120. Results indicate that the preferential expansion of B cells which cross-reactively recognized multiple HIV strains can be achieved by sequentially immunizing mice with different gp120 isolates. Glycosylated gp120 was found to act as a more efficient immunogen than non-glycosylated envelope glycoprotein or V3 look-region specific peptides.

ii) Two murine models of HIV infection are being examined. In the murine AIDS model, levels of T cell activation and lymphokine production are being studied at the single cell level. In transgenic mice expressing gp120 proteins from HIV, the effect of immunization on tolerance and disease progression has been investigated. We are also studying SCID mice reconstituted with human PBL from gp160 vaccinated normal volunteers to analyze the degree of protection conferred by various immunization regimens. An outgrowth of this work has been our finding that anti-HIV reactive cells are present initially in the peripheral cirulation and then home to the bone marrow of immunized donors.

iii) We have been studying the number and antigenic specificity of lymphocytes secreting specific antibodies and lymphokines in patients and mice with SLE and AIDS. Results indicate that two processes - polyclonal activation and (auto) antigen specific immune stimulation - combine to induce the humoral abnormalities found in disease states. Murine models of these diseases are being used to analyze the specificity and cross-reactivity of activated B cells. This work is also providing considerable insight into the degree to which HIV-1 specific B cells are activated in patients with ARC and AIDS.

Publications related to project Z01-04003-04

Klinman DM, Higgins KW, Conover J. Sequential Immunizations with rg120's from independent isolates of HIV-1 induce the preferential expansion of broadly cross-reactive B cells, J Exp Med 1991; 173:881.

Steinberg AD, Gourley MF, Klinman DM, Tsolkos GC, Scott DE, Krieg AM. Pathogenesis of systemic lupus erythematosus, Ann Intern Med 1991; 115:548.

Klinman DM, Shirai A, Ishigatsubo Y, Conover J, Steinberg AD. Quantitation of IgG and IgM secreting B cells in the peripheral blood of patients with systemic lupus erythematosus, Arthritis Rheum 1991; 34:1404.

Klinman DM, Krieg A, Conover J, Ussery MA, Black PL. Effect of cyclophosphamide, total body irradiation and zidovudine on retrovirus proliferation and disease progression in murine AIDS, AIDS Res Hum Retroviruses 1992; 8:101.

Klinman DM. Similarities in B cell repertoire development between autoimmune and aging normal mice, J Immunol 1992; 148:1353.

Shirai A, Leitman-Klinman SF, Klinman DM. HIV infection induces both polyclonal and virus-specific B cell activation, J Clin Invest 1992; 89:561.

Shirai A, Klinman DM. Tolerance abnormalities in systemic lupus erythematosus. In: Hahn BH, Wallace DJ, eds. Dubois' Lupus Erythematosus, 4th Edition. City: Publisher, YEAR;457-72. (in press)

Klinman DM. B cell abnormalities characteristic of systemic lupus erythematosus. In: Hahn BH, Wallace DJ, eds. Dubois' Lupus Erythematosus, 4th Edition. City: Publisher, YEAR;457-72. (in press)

Klinman DM. Analysis of B lymphocyte cross-reactivity at the single cell level, J Immunol Methods. (in press)

Klinman DM, Steimer K, Glass W, Conover J. Comparative immunogenicity of gp120-derived proteins and their induction of anti-V3 loop region antibodies, J AIDS. (in press)

Klinman DM. Viral vectors and potential problems in their use. In: Continuous cell lines-an international workshop on current issues: Dev Biol Stand, 76:299. (in press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 04014-02 LRR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Sera Tested for Neutralizing Antibodies Against Autologous HIV-1 Isolates

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.K. Vujcic, MS, Microbiologist, LRR, DV, CBER
G.V. Quinnan, Jr., MD, Deputy Director/CBER
W.L. Glass, Biologist, LRR, DV, CBER

COOPERATING UNITS *(if any)*

Harvey Alter, MD, NIH Blood Bank
James W. Shih, PhD, Dept of Transfusion Medicine, Clinical Center, NIH

LAB/BRANCH

Laboratory of Retrovirus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Viruses were isolated from each of five different HIV-1 clinical patients on one or as many as three occassions at least six months apart. A total of nine clinical isolates were recovered and each was grown in H9 cells to make a virus pool. Six to ten serum samples from these same five patients were also collected at six month intervals over a period of 2.5-5 years. Sera were tested against their autologous virus isolates to determine if there is a change is neutralizing antibody (NA) titers before and after the virus isolation. No change was evident, however virus isolates from the same patient varied greatly with respect to NA titers. One of the patients that had virus isolated on two occassions one year apart, initially had titers of 1:128-1:256 but the titers against the second isolate were 1:8192-1:16384 which showed that this new virus was easily neutralized. In another patient where three isolates were recovered at six month intervals, the first isolate was highly resistant to neutralization by autologous sera with titers of <1:16, however, the second and third isolate had titers ranging from <1:16-1:64. This data shows that virus isolated from the same patient at different times has distinct patterns of NA activity. These isolates will be further studied by PCR to see if the change in amino acid sequence of the virus envelope glycoprotein can be determined and whether these differences occur within or outside of the V3 loop region of the gp120 which is the region known as the principal neutralizing determinant.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 04015-02 LRR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Preparation of HIV-1 Reference Reagents for the World Health Organization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.K. Vujcic, MS, Microbiologist, LRR, DV, CBER
G.V. Quinnan, Jr., MD, Acting Director/CBER
W.L. Glass, Biologist, LRR, DV, CBER

COOPERATING UNITS *(if any)*

Harvey Alter, MD, NIH Blood Bank
Jackie Melpolder, RN, NIH Blood Bank
Mark Heintzelman, PhD, Chief of Reference Reagents Section, DPQC, CBER

LAB/BRANCH

Laboratory of Retrovirus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Reference sera with neutralizing antibody (NA) titers to HIV-1 were prepared in our laboratory for the World Health Organization (WHO). According to the WHO guidelines for the preparation of reference reagents, a number of procedures and tests are required to assure the quality of the samples. There are 2500-3000 freeze-dried ampules of two different sera ready for labelling and distribution. **Uniformity of fill** of the ampules was achieved for both lots with a standard of deviation of ≤0.012 ml and a coefficient of variation of ≤0.024. **Moisture content** was analyzed using the Gravimetric or Loss on Drying method and was within the appropriate range and **residual oxygen** was not present since the ampules were sealed under vacuum and tested with a high frequency vacuum testing coil to confirm that a vacuum was present. The samples were assessed for **microbial contamination** using fluid thioglycollate media and soybean casein digest media and no growth was observed in either. A short term **stability study** was conducted with 5 samples of each at 56°C, 35°C, 27°C, and 4°C. The results indicated that lyophilized samples placed at 56°C for up to 3 weeks were stable, however at 5 weeks these samples became gelled upon reconstitution. At 35°C, 27°C, and 4°C, samples were stable with no loss in titer for a period of 8 weeks, 5 months, and 7 months, respectively. An **infectivity assay** was done with no detectable virus present at the end of four weeks as determined on a HIV-p24 core profile Elisa test kit. An **international collaborative study** was designed using ten coded samples and six laboratories participated from the U.S. and Europe with each using their own in-house method for determining NA titers. Mean titers were calculated for each of the coded samples which included the reference sera in duplicate for the HIV_{MN}, HIV_{IIIB}, HIV_{RF}, and HIV_{SF2} strains of HIV. Currently, unitage is being decided upon for each of the two samples so that labels can be prepared and the samples can be distributed to WHO centers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 04016-02 LRR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

V3 Loop Region Synthetic Peptides are used to Block HIV-1 Neutralization

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L.K. Vujcic, MS, Microbiologist, LRR, DV, CBER
G.V. Quinnan, Jr., MD, Acting Director/CBER
W.L. Glass, Biologist, LRR, DV, CBER
K.B. Seamon, PhD, Chief, Molecular Pharmacology, DBB, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Retrovirus Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Synthetic peptides of the V3 loop region of HIV-1 envelope glycoprotein were used to measure binding antibodies in an ELISA and as blocking agents in a neutralization assay. Samples from three geographic regions, U.S., Zaire, and Brazil were tested in neutralization assays with peptides representative of a portion of the V3 loop sequence of the HIV_{IIIB}, HIV_{MN}, HIV_{SF2}, and HIV_{Z3} strains to determine if blocking occurs. No peptide blocking was observed using the IIIB and SF2 peptides, minimal blocking was observed using the Z3 peptide and good blocking was observed using the MN peptide. Conclusions about geographic region and peptide blocking relating to strain prevalence in that region are presently being made. Data is currently being summarized in preparation for publication.

Publications related to project Z01 BF 04016 LRR

Carrow EW, Vujcic LK, Glass WL, Seamon SC, Rastogi RM, Hendry R, Boulos N, Nzila N, Quinnan Jr GV. High prevalence of antibodies to the gp120 V3 principal neutralizing determinant of HIV-1_{MN} in sera from Africa and the Americas, AIDS Res Hum Retroviruses 1991; 7:831-838.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 04017-01 LRR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Monkey model for AIDS.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Arifa S. Khan, Supervisory Microbiologist, LRR, DV, CBER

Others	Teresa Galvin	Biologist	LRR, DV, CBER
	Malcolm Martin	Chief	LMM, NIAID, NIH
	Riri Shibata	Visiting Fellow	LMM, NIAID, NIH

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Retrovirus Research

SECTION

Division of Virology

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD. 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.85	0.85	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The human immunodeficiency virus (HIV-1) causes AIDS in man. Its simian counterpart (SIV) induces an AIDS-like disease in Asian monkeys. The development of an animal model system for infection and disease by HIV-1 is essential for AIDS vaccine studies and intervention. Currently, the only animal species which can be infected by HIV-1 is the chimpanzee; however, no disease is produced. Due to the enormous cost of maintaining a chimpanzee and the fact that this cannot be a disease model, a better model was sought. Based upon a recent report from the Washington Primate Research Center, we undertook studies to investigate HIV-1 infection in pig-tail monkeys. Initially, we are testing infectivity of pig-tail PBLs in vitro using various HIV-1 isolates including field isolates (< 4 passages) as well as some laboratory-adapted strains. Since the majority of North American HIV-1 isolates are "MN"-like in the V3 loop of the env region, our aim is to vaccinate and demonstrate protection against an "MN"-like HIV-1 isolate.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 04018-01 LRR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Molecular analysis of mammalian endogenous retroviral sequences.

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: Arifa S. Khan, Supervisory Microbiologist, LRR, DV, CBER

Others:	Theodore Bryan	Microbiologist	LRR, DV, CBER
	Syed Tabriz Ali	Visiting Fellow	LRR, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Retrovirus Researh

SECTION

Division of Virology

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

African monkeys naturally harbor simian immunodeficiency viruses (SIVs), the potential progenitors of human immunodeficiency virus-2 (HIV-2). Asian monkeys can acquire SIV from African monkeys through close contact while in captivity. In addition, an SIV isolated from a chimpanzee has been shown to be closely related to HIV-1, the causative agent of human AIDS. Due to this evolutionary relationship between SIVs and HIVs, there is great concern regarding transmission of the virus from monkey to man via biological products (such as some vaccines) grown in monkey-derived cells. We thus undertook to analyze a variety of monkey DNAs for endogenous SIV/HIV-related sequences which might potentially generate an infectious virus. Nested PCR analysis were carried out on high-molecular-weight DNAs isolated from a Carribbean African green monkey, from Asian monkeys including cynomolgous, pig-tailed, and rhesus and from man. PCR primers were based upon highly conserved sequences in the pol regions of known SIVs and HIVs. Agarose gel analysis of the PCR products indicated amplified fragments from all species. We are currently molecularly cloning these fragments and will obtain their nucleotide sequences to determine the evolutionary relatedness of these amplified endogenous sequences to known infectious SIVs and HIVs.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 05001-04 LHR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Antigenic Structure of Hepatitis A Virus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: S. Feinstone, MD, Chief, LHR, DV, CBER
T. Akatsuka, MD, PhD, Visiting Scientis, LHR, DV, CBER
K. Mihalik, Biologist, LHR, DV, CBER

COOPERATING UNITS *(if any)*

LAB/BRANCH

Laboratory of Hepatitis Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.8	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Immunonologic studies on hepatitis A virus have concentrated on T-cell immunityand experimental vaccinology using the chimpanzee model. Chimpanzees were immunized with the expression product of a recombinant vaccinia virus containing the entire open reading frame of the HAV genome. In appropriate cell culture antigenic particles have been produced. These particles were purified and adsorbed to alum as an experimental vaccine. Two chimpanzees were immunized and one was held as an uninoculated control. Both animals that received the vaccine developed antibody that was apparent even after the first dose. Upon challenge, the uninoculated animal seroconverted but unfortunately did not develope overt hepatitis. The vaccinated animals also appeared to be completely protected but as the control animal was infected without disease it is not possible to prove that they were protected.

Chimpanzee T-cell lines specific for hepatitis A have been established and partially characterized. A cytotoxic T-cell clone has been established and several T-cell lines specific for hepatitis A have been selected. Both cytotoxic and helper activity are being studied and the epitopes to which these cells react are being evaluated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 5002-04 LHR

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Interaction of hepatitis A virus with its cellular receptor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: G. Kaplan, PhD, Senior Research Staff Fellow, LHR, DV, CBER
S. M. Feinstone, MD, Chief, LHR, DV, CBER
A. Dotzauer, PhD, Guest Worker, LHR, DV, CBER
V. Tedeschi, PhD, Guest Worker, LHR, DV, CBER

COOPERATING UNITS *(if any)*

NONE

LAB/BRANCH

Laboratory of Hepatitis Research

SECTION

INSTITUTE AND LOCATION

DV, OBR, CBER, FDA, Bethesda, MD 20892

TOTAL STAFF YEARS: 3.1	PROFESSIONAL: 3.1	OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Hepatitis A virus (HAV), a picornavirus, is the causative agent of an acute hepatic disease; although it is possible to grow HAV in culture and the genome has been molecularly cloned, the general biology of this virus is not well understood. Very little is known about the first step in the life cycle of HAV that is binding to a cell surface receptor which triggers infection. Interaction of HAV with its cellular receptor could be responsible for many characteristics of HAV such as restricted growth in primate cells and hepatovirulence. Therefore, study of the interaction of HAV with its cellular receptor will help to understand the mechanisms involved in the pathogenicity of this virus and specific receptor antagonists could be useful therapeutic agents.

The goal of this project is to study whether presence of specific cell surface receptors are responsible for the restricted growth of HAV and to characterize and molecularly clone cellular receptors for HAV. In order to do so, we are trying to identify nonprimate cell lines capable of suporting HAV replication upon transfection of genomic RNA; cell lines are being transfected with genomic RNA and replication of HAV is being monitored by slot blot hybridization to 32P-labeled cDNA probes. Cell lines that support HAV replication upon transfection will be further analyzed. Specific virus/cell binding

We are also raising monoclonal antibodies against cell surface molecules and analyzing whether such antibodies could protect susceptible cells against HAV infection. Once we obtained such a protective MoAb, we will be able to characterize cell surface molecules that could act as cellular receptors for HAV and molecularly clone them.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 05003-04 LHR

PERIOD COVERED

October 1, 1991 through September 30 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hepatitis C

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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T. Akatsuka, M.D., Ph.D., Visiting Scientist, LHR
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COOPERATING UNITS *(if any)*

A. DiBisceglie, M.D., NIADDK; C. Harris, M.D., NCI; J. Berzofsky, M.D., NCI; W. K. Shih, NIH CC; C. Wychowsky, Ph.D., Institut Pasteur; H. Greenberg, M.D.,Stanford U. Shool of Medicine; C. Rice, Ph.D., Washington U. School of Medicine.

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Laboratory of Hepatitis Research

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
5	4.5	.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research on Hepatitis C (HC) has followed several lines. We have cloned and sequenced the entire genome of HC virus (HCV) and expressed the structural genes in a baculovirus expression system and the entire coding region of the genome in recombinant vaccinia virus. Using the expression products we have isolated human monospecific B cell lines expressing antibody to the HCV core and envelope proteins. The human antibodies to the HCV core protein have been mapped to specific epitopes. One of these antibodies reacts to an epitope near the amino terminus that is well known. The other however, recognizes an epitope near the carboxy terminus that has not been described. We are currently evaluating how common antibody to this epitope is among HCV patients. We also have made mouse monoclonal antibodies to the structural genes, but they are not yet characterized. We have diagnostic assays based on the baculovirus expression products and have shown that up to 30% of chronically infected patients have antibody to one of the surface glycoproteins. We are presently developing an anti-HCV core specific for IgM. We hope to use such an assay to distinguish between patients who have recovered from patients who are asymptomatic but chronically infected. Such an assay may also prove useful in following patients on treatment protocols. We have identified and characterized a T-cell epitope in the NS5 (polymerase) region of the polyprotein. Additional T-cell epitopes are being soufht by several methods and in several regions of the viral polyprotein including stuctural and non-structural proteins. In collaboration with C. Rice we have characterized the NS3 protease and determined the processing of the HCV polyprotein. We have a recombinant vaccinia virus containing the entire open reading frame of the HCV genome which we will evaluate in the chimpanzee model as a potential vaccine. We also have several full length cDNA clones that are being evaluated in transfection systems for infectivity. If one of these clones is infectious it will expand enormously our ability to study and manipulate this virus.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 BF 06001-01 LVVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nuclear localization of the dengue virus capsid protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L. Markoff, MD, Chief, LVVD, DV, CBER
C. Winter, Microbiologist, LVVD, DV, CBER

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The flavivirus capsid protein (C) is a positively charged protein that presumably forms nucleocapsid structures when complexed with virion RNA. At least two forms of capsid exist, that which results from initiation of translation at the most amino-terminal Met in the long ORF in genomic RNA (Cap1) and that which results from initiation of translation at the next downstream Met codon (Cap2). The dengue virus type 4 (DEN4)C can be detected in the nuclei of LLCMK2 cells infected by DEN4 virus. Although flaviviruses are generally thought to replicate in the cytoplasm of infected cells, this result suggests a nuclear involvement in these processes. Proteins that are actively transported from the cytoplasm to the nucleus contain a nuclear localization signal (NLS). The DEN4 C contains several candidate NLS's but the best fit occurs within the 13-amino- acid amino-terminus of Cap1 not included in Cap2. Thus, one would predict that the DEN4 Cap1 but not Cap2 would be transported to the nucleus. To formally identify the NLS in the DEN4 C, Cap1 and Cap2 cDNA were cloned into a vaccinia virus-based expression vector, pTM-1. Using this expression system, we have thus far demonstrated nuclear localization of Cap1 in LLCMK2 cells by IFA in a distribution indistinguishable from that obtained after DEN4 virus infection. Nuclear localiza- tion of Cap2 has thus far not been demonstrated, in keeping with our original hypothesis. Several mutations of the putative NLS in Cap1 have also been generated using PCR. Nuclear localization of these Cap1 mutants remains to be determined but should lead to definitive identification of the NLS. We then plan to assess the requirement for and the effects on the cell of nuclear localization of C during DEN4 virus infection by replacing wild-type C sequences in a full-length genomic cDNA clone with NLS-minus mutant C sequences. Mutant full-length cDNA will be used to generate infectious RNA transcripts in vitro. The phenotype of mutant virus arising from transfection with such transcripts can then be assessed. The effects of C on nuclear function can be assessed using either infectious RNA transcripts or by expression of C using the vaccinia-based system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 06002-01 LVVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Ubiquitination of the dengue virus capsid protein

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6		

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

cDNAs encoding the two putative forms of the DEN4 C, Cap1 and Cap2, were cloned into the pTM-1 expression vector (as reported for project Z01 BF 06001-01 LVVD). Cap1 and Cap2 RNA transcripts were translated in vitro in rabbit reticulocyte lysate, and Cap1 and Cap2 proteins were respectively identified among the products of translation. In addition, higher molecular weight forms of Cap1 and to a lesser extent Cap2 were identifiable and were shown to be ubiquitinated derivatives of the C proteins. Ubiquitin is conjugated to cellular proteins by a covalent linkage to Lys residues in the targeted protein. Ubiquitination of Cap1 was further investigated, since multi-ubiquitination of a Lys residue is known to be linked to ATP-dependent degradation of the substrate, whereas proteins containing mono-ubiquitinated Lys residues are stable. Both Cap1 and Cap2 were shown to be stable in vitro and in vivo in LLCMK2 cells. Since Cap1 contains 12 Lys residues in the 99-amino-acid sequence, and since analysis of our data showed that at least 5 ubiquitin moieties could be linked to Cap1, it was possible that Cap1 was not degraded, because no single Lys residue was multi-ubiquitinated. Analysis of PCR-generated mutants in which N-terminal Lys residues in Cap1 (in positions 5,6, and 16) were changed to Arg suggested that Lys-5 and/or Lys-6 were multi-ubiquitinated. To confirm multi-ubiquitination of Lys-5 or -6, RNA transcripts encoding a carboxy-terminally truncated 46-amino-acid Cap1 mutant protein, including only two additional Lys residues downstream from Lys-5,-6, and -16, were analyzed. Truncated Cap1 molecules containing either Lys or Arg at positions 5,6, and 16 appeared to contain multi-ubiquitinated Lys (evidently at the two available positions downstream from Lys-16) and were degraded in vitro, in contrast to results with full-length Cap1. Future plans include proving that in vitro degradation of truncated Cap1 is related to its ubiquitination, showing that truncated Cap1 is degraded in vivo, and explaining the stability of full-length Cap1. It is possible for example that full length Cap1 is stabilized by an association with another protein which is abrogated by its carboxy-terminal truncation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 06003-06 LVVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of the processing of the flavivirus structural proteins

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: L. Markoff, MD, Chief, LVVD, DV, CBER
B. Falgout, PhD, Senior Staff Fellow, LVVD, DV, CBER

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Studies of the processing of the flavivirus structural proteins, pre-membrane (prM) and envelope (E), previously reported (Project Z01 AI 00501-05), were concluded. prM contains a structurally conserved 35- to 40- amino-acid predominantly hydrophobic domain at its carboxy-terminus which determines the arrest of translocation of prM and the re-initiation of translocation of the polyprotein, as well as the signalase-mediated cleavage at the prM-E site. Hydrophobicity within this domain is assymmetrically interrupted by a conserved Arg residue associated with a short amphipathic segment. We studied the topogenic functions of the 37-amino-acid carboxy-terminus of the dengue virus type 4 (DEN4) prM (residues 243-279 of the DEN4 polyprotein). The 20-amino-acid hydrophobic domain upstream from the conserved Arg (residue 264 in the DEN4 polyprotein) was predicted to constitute the stop-transfer/transmembrane domain for prM (the tm segment). Hydrophobic residues downstream from conserved Arg-264 were predicted to constitute the signal peptide for E. Mutations resulting in the deletion of hydrophobic domains and/or of conserved Arg-264 and the associated amphipathic domain were introduced into DEN4 cDNA. Glycosylation, cleavage at the prM-E and E-NS1 sites, translocation, and membrane integration of wild-type and mutant polypeptides were assessed in vitro. Mutational analysis confirmed that hydrophobic residues 265-279 function as E signal peptide within the larger hydrophobic domain. However, these residues were also necessary but not sufficient for arrest of the translocation of prM and its stable membrane integration, since upstream hydrophobic residues 247-262 were also required for these events to occur. Thus, the topogenic sequences in prM appeared to comprise a novel cleavable signal-anchor (SA) domain not previously described. Results also showed that conserved Arg-264 and the associated amphipathic segment were not required for proper processing of prM and E.

Publications related to project Z01 BF 06003-06 LVVD

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 06004-01 LVVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

An infectious full-length transcript of dengue virus type 2

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

PI: B. Falgout, PH.D., Senior Staff Fellow, LVVD, DV, CBER
Others: L. Markoff, M.D., Chief, LVVD, DV, CBER
S. Polo, Microbiologist, LVVD, DV, CBER

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0.25	0.15	0.1

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The dengue virus genome is a positive-strand RNA molecule of approximately 11 kb, which is capped but not polyadenylated. Purified genomic RNA is infectious upon transfection into appropriate cells. The goal of this project is to clone a cDNA copy of the genome of dengue virus type 2 (DEN2), New Guinea C (NGC) strain. This cDNA will be under control of the prokaryotic SP6 promoter, such that *in vitro* transcription using commercially available SP6 RNA polymerase will generate a faithful copy of the dengue genome. Our specific goal is to produce transcripts that are infectious when transfected into tissue culture cells. Creation of an infectious transcript will allow introduction of defined mutations into the dengue virus genome, which will be useful for basic studies on the life cycle of the virus. Also, there are neurotropic strains of DEN2 NGC that are adapted to growth in mouse brain, as well as strains that are not. By substituting portions of an infectious transcript with cDNA derived from the neurotropic virus, it should be possible to map the mutations responsible for adaptation to growth in mouse brain. Finally, it may be possible to introduce mutations into the genome to attenuate the virus for possible use as a vaccine.

Initially, we attempted to plaque purify DEN2 NGC on LLC-MK$_2$ cells. Although we were able to successfully obtain plaques, our efforts to grow stocks from selected isolated plaques have failed. We have elected to proceed without plaque purification. Stocks of DEN2 NGC have been grown. We are currently designing primers for reverse transcription/polymerase chain reaction. We intend to amplify relatively large pieces of the genome, so that only a few pieces have to be assembled to make a full-length copy.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 BF 06005-01 LVVD

PERIOD COVERED

October 1, 1991 through September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Mutagenesis of the dengue virus protease

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

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Others: Stephanie Polo, Microbiologist, LVVD, DV, CBER

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.55	0.2	0.35

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☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
  ☐ (a1) Minors
  ☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dengue virus encodes a polyprotein that is processed to mature gene products. The majority of the cleavages in the nonstructural (NS) protein region are effected by a virus-encoded protease, consisting of two proteins, NS2B and NS3. NS3 contains the protease active site, but the role of NS2B is less well understood. Recently, we showed that a forty amino acid domain in NS2B is required for protease activity. We have been pursuing two approaches to mutagenize dengue virus type 4 (DEN4) NS2B and NS3, in an effort to better understand this two component protease.

Mutagenesis by selection. Previously, we cloned the DEN4 into the pKK223-3 vector under control of the tac promoter. This plasmid could be grown in *E. coli* JM110 in the absence of IPTG, but in *E. coli* HB101 only very tiny colonies would form. We reasoned that when the protease was expressed it was active and detrimental to *E. coli*. We selected rare large colonies on HB101, sequenced their plasmids, and found that they were all frameshift or nonsense mutations. To try to get around this problem, the lac Z' gene was cloned in-frame downstream of the protease. We transformed JM110 with this plasmid and selected large blue colonies in the presence of IPTG and XGAL. These should have been protease mutants, since they were large, and not frameshift or nonsense, since they were blue and thus expressed the lac Z' fragment. However, sequencing of several such putative mutant plasmids revealed mostly wild-type protease sequences, with two examples of nonsense mutations. We do not understand why the selection is not working as planned.

Site-directed mutagenesis of NS2B. We have had made 20 oligos that can each be used to randomly mutagenize two consecutive residues of the forty amino acid essential domain of NS2B. Mutagenesis is being done in an M13 clone using a commercial kit. Mutants will then have to be subcloned into a pTM1 vector for analysis of their phenotypes. So far, we have had successful mutagenesis reactions with the first two oligos we have tried, and several mutants have been identified and are being subcloned.

PUBLICATIONS

PUBLICATIONS

DIVISION OF VIROLOGY

October 1, 1991 through September 30, 1992

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